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MATERIALS AND METHODS RELATING TO THERAPY AND DIAGNOSIS USING TARGETING OF CELLS THAT EXPRESS JPL POLYPEPTIDES

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CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. Application Serial No. 10/293,244 filed on November 12, 2002, entitled "Novel Nucleic Acids and Polypeptides", Attornmey Docket No. 787CIP4, which is a continuation-in-part of U.S. Application Serial No. 10/258,899 filed on October 29,2002, entitled " Novel Nucleic Acids and Polypeptides", Attorney Docket NO. 787CIP2-2G/US, which is the national phase of International Application Serial No. PCT/US01/04098 filed February 5, 2001, entitled "Novel Nucleic Acids and Polypeptides", Attorney Docket No. 787CIP2-2G/PCT, which is a continuation in-part of U.S. Application Serial No. 09/654,936 filed September 1, 2000 entitled "Novel Nucleic Acids and Polypeptides", Attorney Docket NO. 787CIP2C, which is a continuation-in-part of U.S. Application Serial No. 09/560,875, filed April 27, 2000, entitled "Novel Nucleic Acids and Polypeptides" Attroney Docekt NO. 787CIP, which is a continuation-in-part of U.S. Application Serial NO. 09/496,914 filed February 3, 2000 entitled "Novel Contigs Obtained from Various Libraries", Attorney Docket No. 787, now abandoned; and is a continuation-in-part of a U.S. Application Serial No. 10/459,763, filed on September 27, 2002, entitled "Novel Nucleic Acids and Polypeptides", Attorney Docket NO. 790CIP3/US, which is the national phase of International Application Serial No. PCT/US01/08631, filed March 30, 2001, entitled "Novel Nucleic Acids and Polypeptides", Attorney Docket No. 790CIP3/PCT, which is a continuation-in-part of U.S. Application Serial No. 09/540,217, filed March 31, 2000, entitled "Novel Nucleic Acids and Polypeptides", Attorney Docket No. 790, now abandoned. These and other U.S. Patents and Patent Applications cited herein are hereby incorporated by reference in their entirety.

1. BACKGROUND

1.1 TECHNICAL FIELD

This invention relates to compositions and methods for targeting junctophilin-like-expressing cells using antibodies, polypeptides, polynucleotides, peptides, and small molecules and their use in the therapy and diagnosis of various pathological states including cancers such as melanoma.

1.2 BACKGROUND ART

Immunotherapy provides a method of harnessing the immune system to treat various pathological states, including cancer, autoimmune disease, transplant rejection, hyperproliferative conditions, allergic reactions, emphysema, wound healing, and cardiovascular disease.

Antibody therapy for cancer involves the use of antibodies, or antibody fragments, against a tumor antigen to target antigen-expressing cells. Antibodies, or antibody fragments, may have direct or indirect cytotoxic effects or may be conjugated or fused to cytotoxic moieties. Direct effects include the induction of apoptosis, the blocking of growth factor receptors, and anti-idiotype antibody formation. Indirect effects include antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-mediated cellular cytotoxicity (CMCC). When conjugated or fused to cytotoxic moieties, the antibodies, or fragments thereof, provide a method of targeting the cytotoxicity towards the tumor antigen expressing cells. (Green, *et al.*, *Cancer Treatment Reviews*, 26:269-286 (2000), incorporated herein by reference in its entirety).

Because antibody therapy targets cells expressing a particular antigen, there is a possibility of cross-reactivity with normal cells or tissue. Although some cells, such as hematopoietic cells, are readily replaced by precursors, cross-reactivity with many tissues can lead to detrimental results. Thus, considerable research has gone towards finding tumor-specific antigens. Such antigens are found almost exclusively on tumors or are expressed at a greater level in tumor cells than the corresponding normal tissue. Tumor-specific antigens provide targets for antibody targeting of cancer, or other disease-related cells, expressing the antigen. Antibodies specific to such tumor-specific antigens can be conjugated to cytotoxic compounds or can be used alone in immunotherapy. Immunotoxins target cytotoxic compounds to induce cell death. For example, anti-CD22 antibodies conjugated to deglycosylated ricin A may be used for treatment of B cell

lymphoma that has relapsed after conventional therapy (Amlot, *et al.*, *Blood* 82:2624-2633 (1993), incorporated herein by reference in its entirety) and has demonstrated encouraging responses in initial clinical studies.

The immune system functions to eliminate organisms or cells that are recognized as non-self, including microorganisms, neoplasms and transplants. A cell-mediated host response to tumors includes the concept of immunologic surveillance, by which cellular mechanisms associated with cell-mediated immunity, destroy newly transformed tumor cells after recognizing tumor-associated antigens (antigens associated with tumor cells that are not apparent on normal cells). Furthermore, a humoral response to tumor-associated antigens enables destruction of tumor cells through immunological processes triggered by the binding of an antibody to the surface of a cell, such as antibody-dependent cellular cytotoxicity (ADCC) and complement mediated lysis.

Recognition of an antigen by the immune system triggers a cascade of events including cytokine production, B-cell proliferation, and subsequent antibody production. Often tumor cells have reduced capability of presenting antigen to effector cells, thus impeding the immune response against a tumor-specific antigen. In some instances, the tumor-specific antigen may not be recognized as non-self by the immune system, preventing an immune response against the tumor-specific antigen from occurring. In such instances, stimulation or manipulation of the immune system provides effective techniques of treating cancers expressing one or more tumor-specific antigens.

For example, Rituximab (Rituxan®) is a chimeric antibody directed against CD20, a B cell-specific surface molecule found on >95% of B-cell non-Hodgkin's lymphoma (Press, *et al.*, *Blood* 69:584-591 (1987); Malony, *et al.*, *Blood* 90:2188-2195 (1997), both of which are incorporated herein in their entirety). Rituximab induces ADCC and inhibits cell proliferation through apoptosis in malignant B cells *in vitro* (Maloney, *et al.*, *Blood* 88:637a (1996), incorporated herein by reference in its entirety). Rituximab is currently used as a therapy for advanced stage or relapsed low-grade non-Hodgkin's lymphoma, which has not responded to conventional therapy.

Active immunotherapy, whereby the host is induced to initiate an immune response against its own tumor cells can be achieved using therapeutic vaccines. One type of tumor-specific vaccine uses purified idiotype protein isolated from tumor cells,

coupled to keyhole limpet hemocyanin (KLH) and mixed with adjuvant for injection into patients with low-grade follicular lymphoma (Hsu, *et al.*, *Blood* 89:3129-3135 (1997), incorporated herein by reference in its entirety). Another type of vaccine uses antigen-presenting cells (APCs), which present antigen to naïve T cells during the recognition and effector phases of the immune response. Dendritic cells, one type of APC, can be used in a cellular vaccine in which the dendritic cells are isolated from the patient, co-cultured with tumor antigen and then reinfused as a cellular vaccine (Hsu, *et al.*, *Nat. Med.* 2:52-58 (1996), incorporated herein by reference in its entirety). Immune responses can also be induced by injection of naked DNA. Plasmid DNA that expresses bicistronic mRNA encoding both the light and heavy chains of tumor idiotype proteins, such as those from B cell lymphoma, when injected into mice, are able to generate a protective, anti-tumor response (Singh, *et al.*, *Vaccine* 20:1400-1411 (2002)).

Tumor cells express proteins, such as growth factors, growth factor modulators, and proteases. These proteins play a role in tumor proliferation, differentiation, tissue invasion, metastases and vascularization (angiogenesis). For example, metastasis requires the primary tumor to produce proteases that degrade the extracellular matrix such that tumor cells may enter the blood stream and colonize different tissues. Often these tumor cells will secrete growth or survival factors that will allow them to survive and proliferate in the new tissue. An example of the role of secreted factors in tumorigenesis is demonstrated in some cancers of epithelial origin, such as squamous skin carcinomas, mammary carcinomas, and ovarian adenosarcomas, which can undergo a transient differentiation event called epithelial-to-mesenchymal transformation (EMT) driven by activation of growth factors, such as TGF-β (Thiery and Chopin *Cancer Metastasis Rev.* 18:31-42 (1999), incorporated herein by reference in its entirety). Cells undergoing EMT have altered expression of cell adhesion and cytoskeleton molecules, such as E-cadherin, vinculin, and keratin, and express mesenchymal markers, such as vimentin *de novo* (Oft *et al., Genes Dev.* 10:2462-2477 (1996); Miettinen *et al., J. Cell Biol.* 127:2021-2036 (1994), both of which are incorporated herein by reference in their entirety).

Growth factors are instrumental in inducing angiogenesis as well, which is crucial for tumor growth and invasion. Blood vessels deliver nutrients and oxygen to the tumor

cells and allow tumor cells access to the blood system. TGF-β, for example, induces the expression of the angiogenesis-inducing factor VEGF (Pertovaara *et al., J. Biol. Chem.* 269:6271-6274 (1994), incorporated herein by reference in its entirety) and has indirect effects on angiogenesis as well, such as attracting monocytes that secrete angiogenic cytokines (Sunderkotter *et al., Pharmacol. Ther.* 51:195-216 (1991), incorporated herein by reference in its entirety).

Alternatively, antibodies can be raised against secreted proteins that are involved in regulating processes associated with cancer, such as cell proliferation, differentiation, cell migration, tissue invasion, and angiogenesis. Secreted proteins such as epidermal growth factor (EGF), interleukin-2, and platelet derived growth factor have been demonstrated to play a role in tumor growth, for example. Antibodies can neutralize the activity of the secreted protein by binding to a region that is required for function, for example, a growth factor binding domain, or active site, thereby acting as an inhibitor. Alternatively, antibodies bound to secreted proteins can neutralize antigens by inducing phagocytosis of the antigens by mononuclear phagocytes and neutrophils in a process known as opsonization.

Melanomas are aggressive, frequently metastatic tumors derived from either melanocytes or melanocyte related nevus cells. Even when melanoma is apparently localized to the skin, up to 30% of the patients will develop systemic metastasis and the majority will die (Kirkwood and Agarwala (1993) Principles and Practice of Oncology 7:1-16).

The primary treatment of melanoma is surgical removal. A melanoma that has not spread beyond the site at which it developed is highly curable by surgical excision, and a melanoma that has spread to regional lymph nodes may be curable with wide local excision of the primary tumor and removal of the involved regional lymph nodes. However, a melanoma that has metastasized beyond the regional nodes (American Joint Committee on Cancer stage IV) is a highly lethal disease for which surgical resection is not always possible or helpful. Few affected individuals survive beyond 5 years despite aggressive treatment, which may include chemotherapy, radiation therapy, and adjuvant therapy with interleukin-2 or interferon alpha 2b (Pawlik and Sondak Crit Rev Oncol Hematol 45:245-264 (2003)). In addition, all these treatments are associated with

significant toxicity, and have not been shown to substantially reduce the mortality associated with metastatic disease.

Mounting evidence suggests a prominent role for the immune system in the treatment of melanoma. The use of tumor-associated antigens (TAAs) either by passive immune therapy with antibodies targeted directly to tumor cells or by active immune therapy via vaccination with tumor cells, tumor cell lysates, peptides, carbohydrates, gene constructs encoding proteins, or anti-idiotype antibodies that mimic TAAs, may provide new opportunities for the development of therapeutic strategies against melanoma (Safa and Foon Semin Oncol 28:68-92 (2001)). The success of these novel approaches rests on the discovery of tumor-associated antigens that are specific for the cancer.

Therefore, there exists a need in the art to identify antigens that are clearly and specifically expressed on the surface of melanoma cancer cells that could serve as targets for various immunotherapeutic strategies. Accordingly, Applicants have identified a molecular target useful for therapeutic intervention in melanoma, and provide herein methods for the diagnosis and therapy of melanoma.

2. SUMMARY OF THE INVENTION

This invention is based on the discovery of novel junctophilin-like homolog, including variants, denoted herein as JPL, and the novel isolated polynucleotide encoding such polypeptide, including recombinant DNA molecules, cloned genes or degenerate variants thereof, especially naturally occurring variants such as allelic variants, antisense polynucleotide molecules, and antibodies that specifically recognize one or more epitopes present on such polypeptides, as well as hybridomas producing such antibodies. The compositions of the present invention additionally include vectors such as expression vectors containing the polynucleotides of the invention, cells genetically engineered to contain such polynucleotides, and cells genetically engineered to express such polynucleotides.

The compositions of the invention provide isolated polynucleotides that include, but are not limited to, a polynucleotide comprising any one of the nucleotide sequence set forth in SEQ ID NO: 1, 2, and 3; or a fragment thereof that retains a desired biological activity; and a polynucleotide comprising the full length protein coding sequence of SEQ ID NO: 1,

2, and 3 (for example, SEQ ID NO: 4 and 5). The polynucleotides of the present invention also include, but are not limited to, a polynucleotide that hybridizes under stringent hybridization conditions to anyone of (a) the complement of any of the nucleotide sequences set forth in SEQ ID NO: 1, 2, and 3; (b) a nucleotide sequence encoding any of the amino acid sequences set forth in SEQ ID NO: 4 and 5; a polynucleotide which is an allelic variant of any one of the polynucleotides recited above having at least 70% polynucleotide sequence identity to the polynucleotides; a polynucleotide which encodes a species homolog (e.g. orthologs) of any one of the peptides recited above; or a polynucleotide that encodes a polypeptide comprising a specific domain or truncation of any one of the polypeptides of SEQ ID NO: 4 and 5.

A collection as used in this application can be a collection of only one polynucleotide. The collection of sequence information or unique identifying information of each sequence can be provided on a nucleic acid array. In one embodiment, segments of sequence information are provided on a nucleic acid array to detect the polynucleotide that contains the segment. The array can be designed to detect full-match or mismatch to the polynucleotide that contains the segment. The collection can also be provided in a computer-readable format.

This invention further provides cloning or expression vectors comprising at least a fragment of the polynucleotides set forth above and host cells or organisms transformed with these expression vectors. Useful vectors include plasmids, cosmids, lambda phage derivatives, phagemids, and the like, that are well known in the art. Accordingly, the invention also provides a vector including a polynucleotide of the invention and a host cell containing the polynucleotide. In general, the vector contains an origin of replication functional in at least one organism, convenient restriction endonuclease sites, and a selectable marker for the host cell. Vectors according to the invention include expression vectors, replication vectors, probe generation vectors, and sequencing vectors. A host cell according to the invention can be a prokaryotic or eukaryotic cell and can be a unicellular organism or part of a multicellular organism.

The compositions of the present invention include polypeptides comprising, but not limited to, an isolated polypeptide selected from the group comprising the amino acid sequence of SEQ ID NO: 4 or 5. Polypeptides of the invention also include polypeptides

with biological activity that are encoded by (a) any one of the polynucleotides having a nucleotide sequence set forth in SEQ ID NO: 1, 2 and 3; or (b) polynucleotides that hybridize to the complement of the polynucleotides of (a) under stringent hybridization conditions. Biologically or immunologically active variants of any of the protein sequence listed as SEQ ID NO: 4, and 5 and substantial equivalents thereof that retain biological or immunological activity are also contemplated. The polypeptides of the invention may be wholly or partially chemically synthesized but are preferably produced by recombinant means using the genetically engineered cells (e.g. host cells) of the invention.

The invention also provides compositions comprising a polypeptide of the invention. Pharmaceutical compositions of the invention may comprise a polypeptide of the invention and an acceptable carrier, such as a hydrophilic, e.g., pharmaceutically acceptable, carrier.

The present invention provides a variety of targeting elements and compositions. One such embodiment is a composition comprising an anti-JPL antibody preparation. Exemplary antibodies include a single anti-JPL antibody, a combination of two or more anti-JPL antibodies, a combination of an anti-JPL antibody with a non-JPL antibody, a combination of an anti-JPL antibody and a therapeutic agent, a combination of an anti-JPL antibody and a cytocidal agent, a bispecific anti-JPL antibody, Fab JPL antibodies or fragments thereof, including any fragment of an antibody that retains one or more CDRs that recognize JPL, humanized anti-JPL antibodies that retain all or a portion of a CDR that recognizes a JPL polypeptide, anti-JPL conjugates, and anti-JPL antibody fusion proteins.

Another targeting embodiment of the invention is a vaccine comprising a JPL polypeptide, or a fragment or variant thereof and optionally comprising a suitable adjuvant.

Yet another targeting embodiment is a composition comprising a nucleic acid encoding a JPL polypeptide, or a fragment or variant thereof, optionally within a recombinant vector. A further targeting embodiment of the present invention is a composition comprising an antigen-presenting cell transformed with a nucleic acid encoding a JPL polypeptide, or a fragment or variant thereof, optionally within a recombinant vector. The present invention further provides a method of targeting JPL-expressing cells, which comprises administering a targeting element or composition in an

amount effective to target JPL-expressing cells. Any one of the targeting elements or compositions described herein may be used in such methods, including an anti-JPL antibody preparation, a vaccine comprising a JPL polypeptide, or a fragment or variant thereof or a composition of a nucleic acid encoding JPL, or a fragment or variant thereof, optionally within a recombinant vector or a composition of an antigen-presenting cell transformed with a nucleic acid encoding JPL, or fragment or variant thereof, optionally within a recombinant vector.

Yet another targeting embodiment of the invention is a preparation comprising a JPL polypeptide peptide fragment, or variant thereof. A further targeting embodiment of the present invention is a non-JPL polypeptide or peptide that binds a JPL polypeptide.

Another targeting embodiment of the invention is a preparation comprising a small molecule that binds to a JPL polypeptide.

The invention also relates to methods for producing a polypeptide of the invention comprising culturing host cells comprising an expression vector containing at least a fragment of a polynucleotide encoding the polypeptide of the invention in a suitable culture medium under conditions permitting expression of the desired polypeptide, and purifying the protein or peptide from the culture or from the host cells. Preferred embodiments include those in which the protein produced by such a process is a mature form of the protein.

Polynucleotides according to the invention have numerous applications in a variety of techniques known to those skilled in the art of molecular biology. These techniques include use as hybridization probes, use as oligomers, or primers, for PCR, use in an array, use in computer-readable media, use for chromosome and gene mapping, use in the recombinant production of protein, and use in generation of antisense DNA or RNA, their chemical analogs and the like. For example, when the expression of an mRNA is largely restricted to a particular cell or tissue type, polynucleotides of the invention can be used as hybridization probes to detect the presence of the particular cell or tissue mRNA in a sample using, *e.g., in situ* hybridization.

In other exemplary embodiments, the polynucleotides are used in diagnostics as expressed sequence tags for identifying expressed genes or, as well known in the art and

exemplified by Vollrath et al., Science 258:52-59 (1992), as expressed sequence tags for physical mapping of the human genome.

The polypeptides according to the invention can be used in a variety of conventional procedures and methods that are currently applied to other proteins. For example, a polypeptide of the invention can be used to generate an antibody that specifically binds the polypeptide. Such antibodies, particularly monoclonal antibodies, are useful for detecting or quantitating the polypeptide in tissue.

Methods are also provided for preventing, treating, or ameliorating a medical condition which comprises the step of administering to a mammalian subject a therapeutically effective amount of a composition comprising a peptide of the present invention and a pharmaceutically acceptable carrier.

The methods of the invention also provide methods for the treatment of disorders as recited herein which comprise the administration of a therapeutically effective amount of a composition comprising a polynucleotide or polypeptide of the invention and a pharmaceutically acceptable carrier to a mammalian subject exhibiting symptoms or tendencies related to disorders as recited herein. In addition, the invention encompasses methods for treating diseases or disorders as recited herein comprising the step of administering a composition comprising compounds and other substances that modulate the overall activity of the target gene products and a pharmaceutically acceptable carrier. Compounds and other substances can effect such modulation either on the level of target gene/protein expression or target protein activity. Specifically, methods are provided for preventing, treating or ameliorating a medical condition, including cancer, which comprises administering to a mammalian subject, including but not limited to humans, a therapeutically effective amount of a composition comprising a polypeptide of the invention or a therapeutically effective amount of a composition comprising a binding partner of (e.g., antibody specifically reactive for JPL polypeptides of the invention). The mechanics of the particular condition or pathology will dictate whether the polypeptides of the invention or binding partners (or inhibitors) of these would be beneficial to the individual in need of treatment.

According to this method, polypeptides of the invention can be administered to produce an *in vitro* or *in vivo* inhibition of cellular function. A polypeptide of the

invention can be administered *in vivo* alone or as an adjunct to other therapies. Conversely, protein or other active ingredients of the present invention may be included in formulations of a particular agent to minimize side effects of such an agent.

The invention provides therapeutic and diagnostic methods of targeting cells expressing Junctophilin-like (JPL) proteins by using targeting elements such as JPL polypeptides, nucleic acids encoding JPL protein, and anti-JPL antibodies, including fragments or other modifications thereof. The JPL polypeptide of SEQ ID NO: 5 is highly expressed in melanoma tumors, and in tissue adjacent to the melanoma tumors relative to its expression in healthy organs, including small intestine, spleen, kidney, skeletal muscle, pancreas, lung, liver, lymph node, and heart. Thus, targeting of melanoma cells that express JPL will have a minimal effect on healthy tissues while destroying or inhibiting the growth of the melanoma-based cancer cells. Similarly, other tumors can be targeted if they bear the JPL antigen. For example, inhibition of growth and /or destruction of JPL-expressing cancer cells results from targeting such cells with anti-JPL antibodies. One embodiment of the invention is a method of destroying JPL-expressing melanoma cells by conjugating anti-JPL antibodies with cytocidal materials such as radioisotopes or other cytotoxic compounds.

The present invention provides the use of a variety of targeting elements and compositions to kill or inhibit the growth of JPL-expressing melanoma cancer cells. In one embodiment, the present invention provides the use of a composition that comprises an anti-JPL antibody preparation. Exemplary antibodies include a single anti-JPL antibody, a combination of two or more anti-JPL antibodies, a combination of an anti-JPL antibody with a non-JPL antibody, a combination of anti- JPL antibody and a therapeutic agent, a combination of an anti- JPL antibody and a cytocidal agent, a bispecific anti-JPL antibody, Fab JPL antibodies or fragments thereof, including any fragment of an antibody that retains one or more CDRs that recognize JPL, humanized anti-JPL antibodies that retain all or a portion of a CDR that recognizes JPL, anti-JPL conjugates, and anti- JPL antibody fusion proteins.

Another targeting embodiment of the present invention provides the use of a vaccine for killing or inhibiting the growth of JPL-expressing cells of a melanoma cancer.

The vaccine comprises a JPL polypeptide, or a fragment or variant thereof and optionally comprising a suitable adjuvant.

Yet another targeting embodiment is the use of a composition comprising a nucleic acid encoding JPL, or a fragment or variant thereof, optionally within a recombinant vector in the preparation of a medicament for killing or inhibiting the growth of JPL-expressing melanoma cancer cells. A further targeting embodiment of the present invention is the use of a composition comprising an antigen-presenting cell transformed with a nucleic acid encoding JPL, or a fragment or variant thereof, optionally within a recombinant vector in the preparation of a medicament for killing or inhibiting the growth of JPL-expressing melanoma cancer cells.

The present invention further provides a method of targeting JPL-expressing melanoma cancer cells, which comprises administering a targeting element or composition in an amount effective to target JPL-expressing melanoma cancer cells. Any one of the targeting elements or compositions described herein may be used in such methods, including an anti-JPL antibody preparation, a vaccine comprising a JPL polypeptide, or a fragment or variant thereof or a composition of a nucleic acid encoding JPL, or a fragment or variant thereof, optionally within a recombinant vector or a composition of an antigen-presenting cell transformed with a nucleic acid encoding JPL, or fragment or variant thereof, optionally within a recombinant vector, or a JPL polypeptide, peptide fragment or variant thereof, a binding polypeptide, or peptide that binds to JPL.

The invention also provides a method of killing or inhibiting the growth of melanoma cancer cells, JPL-expressing cancer cells, which comprises administering a targeting element or a targeting composition in an amount effective to inhibit the growth of said cancer cells. Any one of the targeting elements or compositions described herein may be used in such methods, including an anti- JPL antibody preparation, a vaccine comprising a JPL polypeptide, fragment, or variant thereof, composition of a nucleic acid encoding JPL, or fragment or variant thereof, optionally within a recombinant vector, or a composition of an antigen-presenting cell transformed with a nucleic acid encoding JPL, or fragment or variant thereof, optionally within a recombinant vector, or a JPL

polypeptide, peptide fragment, or variant thereof, or a binding polypeptide, or a peptide that binds to JPL.

The present invention further provides a method of treating melanoma in a subject, comprising the step of administering a targeting element or targeting composition in a therapeutically effective amount to treat the melanoma associated with JPL-expressing cells. Any one of the targeting elements or compositions described herein may be used in such methods, including an anti- JPL antibody preparation, a vaccine comprising a JPL polypeptide, fragment, or variant thereof, a composition of a nucleic acid encoding JPL, or fragment or variant thereof, optionally within a recombinant vector, or a composition of an antigen-presenting cell comprising a nucleic acid encoding JPL, or fragment or variant thereof, optionally within a recombinant vector, or a JPL polypeptide, peptide fragment, or variant thereof, or a binding polypeptide, or a peptide that binds to or recognizes a JPL.

In addition to melanoma, other disorders that may be associated with the proliferation of JPL-expressing cells include other cancers, such as Hodgkin's Disease, non-Hodgkin's B-cell lymphomas, T-cell lymphomas, malignant lymphoma, lymphosarcoma leukemia, chronic lymphocytic leukemia, multiple myeloma, acute and chronic myeloid leukemia (also known as myelogenous leukemia), myelomonocytic leukemia, myelodysplastic syndromes, multiple myeloma, X-linked lymphoproliferative disorders; Epstein Barr Virus-related conditions such as mononucleosis; hyperproliferative disorders; autoimmune disorders, such as systemic lupus erythematosus (SLE), Hasimoto thyroiditis, Sjögren's syndrome, pericarditus lupus; wound healing; organ and tissue transplantation rejection (including hyperacute, acute, chronic and xenograft transplant rejection); emphysema; certain allergic reactions; asthma; liver fibrosis; and cardiovascular diseases. Non-hematopoietic tumors that bear the JPL antigen, such as breast, colon, lung, stomach, thymus, epithelial and squamous cell carcinomas, as well as other cancers including gastrointestinal cancers including esophageal cancer, stomach cancer, colon cancer, colorectal cancer, polyps associated with colorectal neoplasms, pancreatic cancer and gallbladder cancer, cancer of the adrenal cortex, ACTH-producing tumor, bladder cancer, brain cancer including intrinsic brain tumors, neuroblastomas, astrocytic brain tumors, gliomas, and metastatic tumor cell

invasion of the central nervous system, Ewing's sarcoma, head and neck cancer including mouth cancer and larynx cancer, kidney cancer including renal cell carcinoma, liver cancer, lung cancer including small and non-small cell lung cancers, malignant peritoneal effusion, malignant pleural effusion, skin cancers, tumor progression of human skin keratinocytes, squamous cell carcinoma, basal cell carcinoma, and hemangiopericytoma, mesothelioma, Kaposi's sarcoma, bone cancer including osteomas and sarcomas such as fibrosarcoma and osteosarcoma, cancers of the female reproductive tract including uterine cancer, endometrial cancer, ovarian cancer, ovarian (germ cell) cancer and solid tumors in the ovarian follicle, vaginal cancer, cancer of the vulva, and cervical cancer; breast cancer (small cell and ductal), penile cancer, prostate cancer, retinoblastoma, testicular cancer, thyroid cancer, trophoblastic neoplasms, and Wilms' tumor, can also be targeted. The invention further provides a method of modulating the immune system by either suppression or stimulation of growth factors and cytokines, by administering the targeting elements or compositions of the invention.

The invention further provides methods for manufacturing medicaments useful in the above-described methods.

The present invention further relates to methods for detecting the presence of the polynucleotides and polypeptides of the invention in a sample (e.g. tissue or sample). Such methods can, for example, be utilized as part of prognostic and diagnostic evaluation of disorders recited herein and for the identification of subjects exhibiting a predisposition to such conditions.

The present invention also provides a method of diagnosing melanoma and disorders associated with JPL–expressing cells comprising the step of measuring the expression patterns of JPL protein and/or its associated mRNA. Yet another embodiment of a method of diagnosing disorders associated with JPL-expressing cells comprises the step of detecting JPL expression using anti-JPL antibodies. Expression levels or patterns may then be compared with a suitable standard indicative of the desired diagnosis. Such methods of diagnosis include compositions, kits and other approaches for determining whether a patient is a candidate for JPL therapy in which said JPL is targeted.

The invention provides a method for detecting a polypeptide of the invention in a sample comprising contacting the sample with a compound that binds to and forms a

complex with the polypeptide under conditions and for a period sufficient to form the complex and detecting formation of the complex, so that if a complex is formed, the polypeptide is detected.

The invention also provides kits comprising polynucleotide probes and/or monoclonal antibodies, and optionally quantitative standards, for carrying out methods of the invention. Furthermore, the invention provides methods for evaluating the efficacy of drugs, and monitoring the progress of patients, involved in clinical trials for the treatment of disorders as recited above.

The invention also provides methods for the identification of compounds (i.e. peptides, or small molecules) that modulate (i.e., increase or decrease) the expression or activity of the polynucleotides and/or polypeptides of the invention. Such methods can be utilized, for example, for the identification of compounds that can ameliorate symptoms of disorders as recited herein. Such methods can include, but are not limited to, assays for identifying compounds and other substances that interact with (*e.g.*, bind to) the polypeptides of the invention.

The invention provides a method for identifying a compound that binds to the polypeptide of the present invention comprising contacting the compound with the polypeptide under conditions and for a time sufficient to form a polypeptide/compound complex and detecting the complex, so that if the polypeptide/compound complex is detected, a compound that binds to the polypeptide is identified.

Also provided is a method for identifying a compound that binds to the polypeptide comprising contacting the compound with the polypeptide in a cell for a time sufficient to form a polypeptide/compound complex wherein the complex drives expression of a reporter gene sequence in the cell and detecting the complex by detecting reporter gene sequence expression so that if the polypeptide/compound complex is detected a compound that binds to the polypeptide is identified.

The present invention also provides a method of enhancing the effects of therapeutic agents and adjunctive agents used to treat and manage melanoma and disorders associated with JPL-expressing cells, by administering JPL preparations with therapeutic and adjuvant agents commonly used to treat such disorders.

3. BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the nucleic acid sequence of a cDNA (SEQ ID NO: 3) encoding a JPL polypeptide.

Figure 2 depicts the amino acid sequence of the JPL polypeptide (SEQ ID NO: 5) encoded by the polynucleotide of Figure 1.

Figure 3 shows a CLUSTALW alignment of the JPL polypeptide of SEQ ID: 5, junctophilin-1 (JP-1; SEQ ID NO: 6), junctophilin-2 (JP-2; SEQ ID NO: 7), and junctophilin-3 (JP-3; SEQ ID NO: 8).

Figure 4 shows the expression of JPL mRNA derived from melanomas and tissue adjacent to the melanomas relative to the expression of JPL in healthy organs.

4. DETAILED DESCRIPTION OF THE INVENTION

JPL polypeptides and polynucleotides encoding such polypeptides are disclosed in co-owned U.S. Patent Applications Serial No. 09/654,936 (corresponding to PCT Publication No. WO 01/04098), 10/258,899 (corresponding to PCT publication WO 01/57190) and 10/293,244. These and all other US patents and patent applications, foreign patents and PCT publications cited herein are hereby incorporated by reference in their entirety. U.S. Application Serial Nos. 10/302,689, 09/496,914 and 09/540,217 incorporated by reference in their entirety relates in general to a collection or library of at least one novel nucleic acid sequences, specifically contigs, assembled from expressed sequence tags (ESTs). U.S. Patent Application Serial Nos. 09/654,936, 10/258,899, and 10/293,244 which are herein incorporated by reference in their entirety, (specifically including all sequences in the sequence listing) disclose JPL polypeptides, isolated polynucleotides encoding such polypeptides, including recombinant molecules, cloned genes or degenerate variants thereof, especially naturally occurring variants such as allelic variants, fragments or analogs or variants of such polynucleotides or polypeptides, antisense polynucleotide molecules, and antibodies that specifically recognize one or more epitopes present on such polypeptides, including polyclonal, monoclonal, single chain, bispecific, fragment, human and humanized antibodies, as well as hybridomas producing monoclonal antibodies, and diagnostic and therapeutic uses and screening assays associated with such polynucleotides, polypeptides and antibodies. These and all

other U.S. patents and patent applications, foreign patents and PCT publications cited herein are hereby incorporated by reference in their entirety.

The JPL polypeptide of SEQ ID NO: 5 is an approximately 628 amino acid protein with a predicted molecular mass of 69 kDa unglycosylated. Using the TMpred program (K. Hofmann & W. Stoffel Biol Chem Hoppe-Seyler 374: 166-170 (1993)), SEQ ID NO: 5 is predicted to have a transmembrane domain at approximately residue 607 to residue 623 (SEQ ID NO: 9). One skilled in the art will recognize that the actual domain may be different that that predicted by the computer program.

Using eMATRIX software package (Stanford University, Stanford, CA) (Wu et al., J. Comp. Biol., vol. 6, pp. 219-235 (1999), herein incorporated by reference), the JPL polypeptide of SEQ ID NO: 5 is expected to have a complement C1q domain (RRTSLDSGHSDPPTPPPPLPLPGDEGGSPASGSRG designated as SEQ ID NO: 10; p-value 9.86E-9, IPB001073A (identification number correlating to signature)) at residues 159-193 of SEQ ID NO: 5 wherein wherein A=Alanine, C=Cysteine, D=Aspartic Acid, E= Glutamic Acid, F=Phenylalanine, G=Glycine, H=Histidine, I=Isoleucine, K=Lysine, L=Leucine, M=Methionine, N=Asparagine, P=Proline, Q=Glutamine, R=Arginine, S=Serine, T=Threonine, V=Valine, W=Tryptophan, Y=Tyrosine.

Using the pfam families database (Washington University School of Medicine, St. Louis, MO; Bateman et al., Nucl Acids Res 30:276-280 (2002), herein incorporated by reference in its entirety, JPL is predicted to contain 8 MORN domains at residues 15-33, 39-60, 61-77, 83-105, 106-128, 129-143, 282-304, and 305-327 (accession number PF02493).

Protein database searches with the BLASTP algorithm (Altschul S.F. et al., J. Nucleic Acid Res 25:3389-3402 (1997), herein incorporated by reference) indicate that the JPL polypeptide of SEQ ID NO: 5 is homologous to junctophilins subtypes 1, 2, and 3 (JP-1, JP-2, JP-3), and shares the highest identity with JP-3. Figure 3 shows a CLUSTLW amino acid sequence alignment between JPL (SEQ ID NO: 5), and the human junctophilins JP-1 (accession number gi 21735575; SEQ ID NO: 6), JP-2 (accession no. 21704281; SEQ ID NO: 7), and JP-3 (accession no. 9886738; SEQ ID NO: 8), wherein A=Alanine, C=Cysteine, D=Aspartic Acid, E= Glutamic Acid, F=Phenylalanine, G=Glycine, H=Histidine, I=Isoleucine, K=Lysine, L=Leucine,

M=Methionine, N=Asparagine, P=Proline, Q=Glutamine, R=Arginine, S=Serine, T=Threonine, V=Valine, W=Tryptophan, Y=Tyrosine. Analysis of global pairwise alignments between JPL and JP-1, JP-2 or JP-3 determined that the percent identity and similarity between JPL and JP-1 are 42% and 58%, respectively; the percent identity and similarity between JPL and JP-2 are 44% and 58% respectively; and the percent identity and similarity between JPL and JP-3 are 44% and 57% (Needleman, S.B. and Wunsch, Ch.D. J Mol Biol 48:443–453 (1970)). The analysis determines the percent identity and similarity over the entire polypeptide sequence.

The junctophilins (JPs) JP-1, JP-2, and JP-3 constitute a family of junctional membrane complex proteins that span the membrane of the intracellular Ca 2+ store and interact with the cell-surface membrane. JPs are composed of a large cytoplasmic region and a carboxyl-terminal transmembrane segment spanning the endoplasmic/sarcoplasmic reticulum. The cytoplasmic regions of JPs contain 8 of the 14 amino acid residue MORN motif through which they exhibit selective binding affinity to the plasma membrane (Nishi et al., Biochem Biophys Res Commun 273:920-927 (2000), incorporated herein by reference in its entirety).

JPs exhibit a high degree of structural conservation across species, and have been identified in human, mouse, and rabbit Nishi et al., Biochem Biophys Res Commun 273:920-927 (2000)). Species homologs of JPs are also found in the invertebrates C. Elegans and D. melanogaster (accession numbers AB069909 and AB069908, respectively) (Yoshida et al., Biochem Biophys Res Commun 289:234-239 (2001), incorporated herein by reference in its entirety).

Junctophilins have been identified in excitable tissues: JP-1 is found predominantly in skeletal muscle, JP-2 is expressed throughout skeletal, cardiac and smooth muscle cells, and JP-3 is expressed specifically in the brain (Takeshima et al., Mol Cell 6:11-22 (2000); Nishi et al., BBRC 292:318-324 (2002), incorporated herein by reference in its entirety). JP-1 and JP-2 play important roles in the formation of triads and diads, respectively, during the development of skeletal muscle in mouse (Ito et al., J Cell Biol 154:1059-1068 (2001); Komazaki et al., FEBS Lett 524:225-229 (2002), incorporated herein by reference in thei entirety), and JP-2 is also essential for the physiological coupling in cardiac myocytes between the dyhudropyridine receptor and

the ryanodine receptor (Yoshida et al., Biochem Biophys Res Commun 289:234-239 (2001), incorporated herein by reference in its entirety). Mice lacking JP-2 exhibit embryonic lethality due to cardiac failure (Takeshima et al., Mol Cell 6: 11-22 (2000). Triplet repeat expansions of the JP-3 gene are associated with Huntigton disease like symptoms including motor disorder in humans (Margolis et al., Nature Genetics29:377-378 (2001); Stevanin et al., Brain 126:1599-1603 (2003), incorporated herein by reference in their entirety), and mice lacking JP-3 have impaired balance/motor coordination (Nishi et al., Biochem Biophys Res commun 292:318-324 (2002)).

The polypeptide of the invention (SEQ ID NO: 5) has a tissue distribution that is most similar to that of JP-3. Consistent with the findings of Takeshima et al. and Nishi et al. regarding the tissue distribution of JP-3, Applicants have shown that that JPL mRNA is expressed abundantly in normal brain tissue, but is absent in other normal adult organs (Figure 4). In addition, Applicants have discovered that JPL mRNA is expressed at high levels in melanoma tumor tissue and in tissue adjacent to the melanoma tumors (Figure 4). The restricted pattern of expression of JPL indicates that JPL is an antigen that is suitable for a variety of targeting strategies.

The present invention relates to methods of targeting melanoma cancer cells that express JPL using targeting elements, such as JPL polypeptides, nucleic acids encoding JPL, anti-JPL antibodies, including fragments or other modifications of any of these elements. The brain is the only healthy organ that expresses JPL (Figure 4, Example 1), and since the targeting elements of the invention cannot cross the blood-brain barrier, targeting of JPL-expressing cancer cells would not compromise the biological function of JPL in the brain.

The present invention provides a novel approach for diagnosing and treating melanoma and disorders associated with JPL-expressing cells. The method comprises administering an effective dose of targeting preparations such as vaccines, antigen presenting cells, or pharmaceutical compositions comprising the targeting elements, JPL polypeptides, nucleic acids encoding JPL, anti-JPL antibodies, described below. Targeting of JPL on the cell membranes of JPL-expressing cells is expected to inhibit the growth of or destroy such cells. An effective dose will be the amount of such targeting

JPL preparations necessary to target JPL on the cell membrane and inhibit the growth of or destroy the JPL-expressing cells and/or metastasis.

A further embodiment of the present invention is to enhance the effects of therapeutic agents and adjunctive agents used to treat and manage disorders associated with JPL–expressing cells, by administering JPL preparations with therapeutic and adjuvant agents commonly used to treat such disorders. Chemotherapeutic agents useful in treating neoplastic disease and antiproliferative agents and drugs used for immunosuppression include alkylating agents, such as nitrogen mustards, alkyl sulfonates, nitrosoureas, triazenes; antimetabolites, such as folic acid analogs, pyrimidine analogs, and purine analogs; natural products, such as vinca alkaloids, epipodophyllotoxins, antibiotics, and enzymes; miscellaneous agents such as polatinum coordination complexes, substituted urea, methyl hydrazine derivatives, and adrenocortical suppressant; and hormones and antagonists, such as adrenocorticosteroids, progestins, estrogens, androgens, and anti-estrogens (Calebresi and Parks, pp. 1240-1306 in, Eds. A.G Goodman, L.S. Goodman, T.W. Rall, and F. Murad, *The Pharmacological Basis of Therapeutics*, Seventh Edition, MacMillan Publishing Company, New York, (1985), incorporated herein by reference in their entirety).

Adjunctive therapy used in the management of such disorders includes, for example, radiosensitizing agents, coupling of antigen with heterologous proteins, such as globulin or beta-galactosidase, or inclusion of an adjuvant during immunization.

High doses may be required for some therapeutic agents to achieve levels to effectuate the target response, but may often be associated with a greater frequency of dose-related adverse effects. Thus, combined use of the immunotherapeutic methods of the present invention with agents commonly used to treat JPL protein-related melanoma allows the use of relatively lower doses of such agents resulting in a lower frequency of adverse side effects associated with long-term administration of the conventional therapeutic agents. Thus another indication for the immunotherapeutic methods of this invention is to reduce adverse side effects associated with conventional therapy of melanoma associated with JPL-expressing cells.

4.1 DEFINITIONS

The term "melanoma" includes, but is not limited to, melanomas, metastatic melanomas, melanomas derived from either melanocytes or melanocytes related nevus cells, melanocarcinomas, melanoepitheliomas, melanosarcomas, melanoma in situ, superficial spreading melanoma, nodular melanoma, lentigo maligna melanoma, acral lentiginous melanoma, invasive melanoma or familial atypical mole and melanoma (FAM-M) syndrome. Such melanomas in mammals may be caused by, chromosomal abnormalities, degenerative growth and developmental disorders, mitogenic agents, ultraviolet radiation (UV), viral infections, inappropriate tissue expression of a gene, alterations in expression of a gene, or carcinogenic agents. The aforementioned melanomas can be diagnosed, assessed or treated by methods described in the present application.

The term "active" refers to those forms of the polypeptide that retain the biologic and/or immunologic activities of any naturally occurring polypeptide. According to the invention, the terms "biologically active" or "biological activity" refer to a protein or peptide having structural, regulatory or biochemical functions of a naturally occurring molecule. Likewise "biologically active" or "biological activity" refers to the capability of the natural, recombinant or synthetic JPL peptide, or any peptide thereof, to induce a specific biological response in appropriate animals or cells and to bind with specific antibodies. The term "JPL biological activity" refers to biological activity that is similar to the biological activity of a JPL polypeptide.

The term "activated cells" as used in this application are those cells which are engaged in extracellular or intracellular membrane trafficking, including the export of secretory or enzymatic molecules as part of a normal or disease process.

The terms "complementary" or "complementarity" refer to the natural binding of polynucleotides by base pairing. For example, the sequence 5'-AGT-3' binds to the complementary sequence 3'-TCA-5'. Complementarity between two single-stranded molecules may be "partial" such that only some of the nucleic acids bind or it may be "complete" such that total complementarity exists between the single stranded molecules. The degree of complementarity between the nucleic acid strands has significant effects on the efficiency and strength of the hybridization between the nucleic acid strands.

The term "embryonic stem cells (ES)" refers to a cell that can give rise to many differentiated cell types in an embryo or an adult, including the germ cells. The term "germ line stem cells (GSCs)" refers to stem cells derived from primordial stem cells that provide a steady and continuous source of germ cells for the production of gametes. The term "primordial germ cells (PGCs)" refers to a small population of cells set aside from other cell lineages particularly from the yolk sac, mesenteries, or gonadal ridges during embryogenesis that have the potential to differentiate into germ cells and other cells. PGCs are the source from which GSCs and ES cells are derived The PGCs, the GSCs and the ES cells are capable of self-renewal. Thus these cells not only populate the germ line and give rise to a plurality of terminally differentiated cells that comprise the adult specialized organs, but are able to regenerate themselves. The term "totipotent" refers to the capability of a cell to differentiate into all of the cell types of an adult organism. The term "pluripotent" refers to the capability of a cell to differentiate into a number of differentiated cell types that are present in an adult organism. A pluripotent cell is restricted in its differentiation capability in comparison to a totipotent cell.

The term "expression modulating fragment," EMF, means a series of nucleotides that modulates the expression of an operably linked ORF or another EMF.

As used herein, a sequence is said to "modulate the expression of an operably linked sequence" when the expression of the sequence is altered by the presence of the EMF. EMFs include, but are not limited to, promoters, and promoter modulating sequences (inducible elements). One class of EMFs is nucleic acid fragments which induce the expression of an operably linked ORF in response to a specific regulatory factor or physiological event.

The terms "nucleotide sequence" or "nucleic acid" or "polynucleotide" or "oligonculeotide" are used interchangeably and refer to a heteropolymer of nucleotides or the sequence of these nucleotides. These phrases also refer to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA) or to any DNA-like or RNA-like material. In the sequences, A is adenine, C is cytosine, G is guanine, and T is thymine, while N is A, T, G, or C. It is contemplated that where the polynucleotide is RNA, the T (thymine) in the sequence herein may be replaced with U (uracil). Generally,

nucleic acid segments provided by this invention may be assembled from fragments of the genome and short oligonucleotide linkers, or from a series of oligonucleotides, or from individual nucleotides, to provide a synthetic nucleic acid which is capable of being expressed in a recombinant transcriptional unit comprising regulatory elements derived from a microbial or viral operon, or a eukaryotic gene.

The terms "oligonucleotide fragment" or a "polynucleotide fragment", "portion," or "segment" or "probe" or "primer" are used interchangeably and refer to a sequence of nucleotide residues which are at least about 5 nucleotides, more preferably at least about 7 nucleotides, more preferably at least about 9 nucleotides, more preferably at least about 11 nucleotides and most preferably at least about 17 nucleotides. The fragment is preferably less than about 500 nucleotides, preferably less than about 200 nucleotides, more preferably less than about 100 nucleotides, more preferably less than about 50 nucleotides and most preferably less than 30 nucleotides. Preferably the probe is from about 6 nucleotides to about 200 nucleotides, preferably from about 15 to about 50 nucleotides, more preferably from about 17 to 30 nucleotides and most preferably from about 20 to 25 nucleotides. Preferably the fragments can be used in polymerase chain reaction (PCR), various hybridization procedures or microarray procedures to identify or amplify identical or related parts of mRNA or DNA molecules. A fragment or segment may uniquely identify each polynucleotide sequence of the present invention. Preferably the fragment comprises a sequence substantially similar to a portion of SEQ ID NO: 1, 2, or 3.

Probes may, for example, be used to determine whether specific mRNA molecules are present in a cell or tissue or to isolate similar nucleic acid sequences from chromosomal DNA as described by Walsh et al. (Walsh, P.S. et al., 1992, PCR Methods Appl 1:241-250). They may be labeled by nick translation, Klenow fill-in reaction, PCR, or other methods well known in the art. Probes of the present invention, their preparation and/or labeling are elaborated in Sambrook, J. et al., 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, NY; or Ausubel, F.M. et al., 1989, Current Protocols in Molecular Biology, John Wiley & Sons, New York NY, both of which are incorporated herein by reference in their entirety.

The nucleic acid sequences of the present invention also include the sequence information from any of the nucleic acid sequences of SEQ ID NO: 1, 2, or 3. The sequence information can be a segment of SEQ ID NO: 1, 2, or 3 that uniquely identifies or represents the sequence information of SEQ ID NO: 1, 2, or 3. One such segment can be a twenty-mer nucleic acid sequence because the probability that a twenty-mer is fully matched in the human genome is 1 in 300. In the human genome, there are three billion base pairs in one set of chromosomes. Because 4^{20} possible twenty-mers exist, there are 300 times more twenty-mers than there are base pairs in a set of human chromosomes. Using the same analysis, the probability for a seventeen-mer to be fully matched in the human genome is approximately 1 in 5. When these segments are used in arrays for expression studies, fifteen-mer segments can be used. The probability that the fifteen-mer is fully matched in the expressed sequences is also approximately one in five because expressed sequences comprise less than approximately 5% of the entire genome sequence.

Similarly, when using sequence information for detecting a single mismatch, a segment can be a twenty-five mer. The probability that the twenty-five mer would appear in a human genome with a single mismatch is calculated by multiplying the probability for a full match ($1 \div 4^{25}$) times the increased probability for mismatch at each nucleotide position (3 x 25). The probability that an eighteen mer with a single mismatch can be detected in an array for expression studies is approximately one in five. The probability that a twenty-mer with a single mismatch can be detected in a human genome is approximately one in five.

The term "open reading frame," ORF, means a series of nucleotide triplets coding for amino acids without any termination codons and is a sequence translatable into protein.

The terms "operably linked" or "operably associated" refer to functionally related nucleic acid sequences. For example, a promoter is operably associated or operably linked with a coding sequence if the promoter controls the transcription of the coding sequence. While operably linked nucleic acid sequences can be contiguous and in the same reading frame, certain genetic elements e.g. repressor genes are not contiguously linked to the coding sequence but still control transcription/translation of the coding sequence.

The term "pluripotent" refers to the capability of a cell to differentiate into a number of differentiated cell types that are present in an adult organism. A pluripotent cell is restricted in its differentiation capability in comparison to a totipotent cell.

The terms "polypeptide" or "peptide" or "amino acid sequence" refer to an oligopeptide, peptide, polypeptide or protein sequence or fragment thereof and to naturally occurring or synthetic molecules. A polypeptide "fragment," "portion," or "segment" is a stretch of amino acid residues of at least about 5 amino acids, preferably at least about 7 amino acids, more preferably at least about 9 amino acids and most preferably at least about 17 or more amino acids. The peptide preferably is not greater than about 200 amino acids, more preferably less than 150 amino acids and most preferably less than 100 amino acids. Preferably the peptide is from about 5 to about 200 amino acids. To be active, any polypeptide must have sufficient length to display biological and/or immunological activity.

The term "naturally occurring polypeptide" refers to polypeptides produced by cells that have not been genetically engineered and specifically contemplates various polypeptides arising from post-translational modifications of the polypeptide including, but not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation.

The term "translated protein coding portion" means a sequence which encodes for the full length protein which may include any leader sequence or a processing sequence.

The term "mature protein coding sequence" refers to a sequence which encodes a peptide or protein without any leader/signal sequence. The "mature protein portion" refers to that portion of the protein without the leader/signal sequence. The peptide may have the leader sequences removed during processing in the cell or the protein may have been produced synthetically or using a polynucleotide only encoding for the mature protein coding sequence. It is contemplated that the mature protein portion may or may not include an initial methionine residue. The initial methionine is often removed during processing of the peptide.

The term "derivative" refers to polypeptides chemically modified by such techniques as ubiquitination, labeling (e.g., with radionuclides or various enzymes), covalent polymer attachment such as pegylation (derivatization with polyethylene glycol)

and insertion or substitution by chemical synthesis of amino acids such as ornithine, which do not normally occur in human proteins.

The term "variant"(or "analog") refers to any polypeptide differing from naturally occurring polypeptides by amino acid insertions, deletions, and substitutions, created using, *e g.*, recombinant DNA techniques. Guidance in determining which amino acid residues may be replaced, added or deleted without abolishing activities of interest, may be found by comparing the sequence of the particular polypeptide with that of homologous peptides and minimizing the number of amino acid sequence changes made in regions of high homology (conserved regions) or by replacing amino acids with consensus sequence.

Alternatively, recombinant variants encoding these same or similar polypeptides may be synthesized or selected by making use of the "redundancy" in the genetic code. Various codon substitutions, such as the silent changes which produce various restriction sites, may be introduced to optimize cloning into a plasmid or viral vector or expression in a particular prokaryotic or eukaryotic system. Mutations in the polynucleotide sequence may be reflected in the polypeptide or domains of other peptides added to the polypeptide to modify the properties of any part of the polypeptide, to change characteristics such as ligand-binding affinities, interchain affinities, or degradation/turnover rate.

Preferably, amino acid "substitutions" are the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, *i.e.*, conservative amino acid replacements. "Conservative" amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid. "Insertions" or "deletions" are preferably in the range of about 1 to 20 amino acids, more preferably 1 to 10 amino acids. The variation allowed may be experimentally determined by systematically making insertions,

deletions, or substitutions of amino acids in a polypeptide molecule using recombinant DNA techniques and assaying the resulting recombinant variants for activity.

Alternatively, where alteration of function is desired, insertions, deletions or non-conservative alterations can be engineered to produce altered polypeptides. Such alterations can, for example, alter one or more of the biological functions or biochemical characteristics of the polypeptides of the invention. For example, such alterations may change polypeptide characteristics such as ligand-binding affinities, interchain affinities, or degradation/turnover rate. Further, such alterations can be selected so as to generate polypeptides that are better suited for expression, scale up and the like in the host cells chosen for expression. For example, cysteine residues can be deleted or substituted with another amino acid residue in order to eliminate disulfide bridges.

The term "immunogenic" refers to the capacity of the natural, recombinant or synthetic JPL polypeptide, or any peptide thereof, to induce a specific immune response in appropriate animals or cells, and to bind with specific antibodies.

The terms "purified" or "substantially purified" as used herein denotes that the indicated nucleic acid or polypeptide is present in the substantial absence of other biological macromolecules, *e.g.*, polynucleotides, proteins, and the like. In one embodiment, the polynucleotide or polypeptide is purified such that it constitutes at least 95% by weight, more preferably at least 99% by weight, of the indicated biological macromolecules present (but water, buffers, and other small molecules, especially molecules having a molecular weight of less than 1000 daltons, can be present).

The term "isolated" as used herein refers to a nucleic acid or polypeptide separated from at least one other component (e.g., nucleic acid or polypeptide) present with the nucleic acid or polypeptide in its natural source. In one embodiment, the nucleic acid or polypeptide is found in the presence of (if anything) only a solvent, buffer, ion, or other components normally present in a solution of the same. The terms "isolated" and "purified" do not encompass nucleic acids or polypeptides present in their natural source.

The term "recombinant," when used herein to refer to a polypeptide or protein, means that a polypeptide or protein is derived from recombinant (*e.g.*, microbial, insect, or mammalian) expression systems. "Microbial" refers to recombinant polypeptides or proteins made in bacterial or fungal (e.g., yeast) expression systems. As a product,

"recombinant microbial" defines a polypeptide or protein essentially free of native endogenous substances and unaccompanied by associated native glycosylation. Polypeptides or proteins expressed in most bacterial cultures, *e.g., E. coli*, will be free of glycosylation modifications; polypeptides or proteins expressed in yeast will have a glycosylation pattern in general different from those expressed in mammalian cells.

The term "recombinant expression vehicle or vector" refers to a plasmid or phage or virus or vector, for expressing a polypeptide from a DNA (RNA) sequence. An expression vehicle can comprise a transcriptional unit comprising an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, promoters or enhancers, (2) a structural or coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate transcription initiation and termination sequences. Structural units intended for use in yeast or eukaryotic expression systems preferably include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, where recombinant protein is expressed without a leader or transport sequence, it may include an amino terminal methionine residue. This residue may or may not be subsequently cleaved from the expressed recombinant protein to provide a final product.

The term "recombinant expression system" means host cells which have stably integrated a recombinant transcriptional unit into chromosomal DNA or carry the recombinant transcriptional unit extrachromosomally. Recombinant expression systems as defined herein will express heterologous polypeptides or proteins upon induction of the regulatory elements linked to the DNA segment or synthetic gene to be expressed. This term also means host cells which have stably integrated a recombinant genetic element or elements having a regulatory role in gene expression, for example, promoters or enhancers. Recombinant expression systems as defined herein will express polypeptides or proteins endogenous to the cell upon induction of the regulatory elements linked to the endogenous DNA segment or gene to be expressed. The cells can be prokaryotic or eukaryotic.

The term "secreted" includes a protein that is transported across or through a membrane, including transport as a result of signal sequences in its amino acid sequence when it is expressed in a suitable host cell. "Secreted" proteins include without limitation

proteins secreted wholly (*e.g.*, soluble proteins) or partially (*e.g.*, receptors) from the cell in which they are expressed. "Secreted" proteins also include without limitation proteins that are transported across the membrane of the endoplasmic reticulum. "Secreted" proteins are also intended to include proteins containing non-typical signal sequences (e.g. Interleukin-1 Beta, see Krasney, P.A. and Young, P.R. (1992) Cytokine 4(2):134 - 143) and factors released from damaged cells (e.g. Interleukin-1 Receptor Antagonist, see Arend, W.P. et. al. (1998) Annu. Rev. Immunol. 16:27-55)

Where desired, an expression vector may be designed to contain a "signal or leader sequence" which will direct the polypeptide through the membrane of a cell. Such a sequence may be naturally present on the polypeptides of the present invention or provided from heterologous protein sources by recombinant DNA techniques.

The term "stringent" is used to refer to conditions that are commonly understood in the art as stringent. Stringent conditions can include highly stringent conditions (i.e., hybridization to filter-bound DNA in 0.5 M $NaHPO_4$, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C, and washing in 0.1X SSC/0.1% SDS at 68°C), and moderately stringent conditions (i.e., washing in 0.2X SSC/0.1% SDS at 42°C). Other exemplary hybridization conditions are described herein in the examples.

In instances of hybridization of deoxyoligonucleotides, additional exemplary stringent hybridization conditions include washing in 6X SSC/0.05% sodium pyrophosphate at 37°C (for 14-base oligonucleotides), 48°C (for 17-base oligonucleotides), 55°C (for 20-base oligonucleotides), and 60°C (for 23-base oligonucleotides).

As used herein, "substantially equivalent" can refer both to nucleotide and amino acid sequences, for example a mutant sequence, that varies from a reference sequence by one or more substitutions, deletions, or additions, the net effect of which does not result in an adverse functional dissimilarity between the reference and subject sequences. Typically, such a substantially equivalent sequence varies from one of those listed herein by no more than about 35% (*i.e.*, the number of individual residue substitutions, additions, and/or deletions in a substantially equivalent sequence, as compared to the corresponding reference sequence, divided by the total number of residues in the substantially equivalent sequence is about 0.35 or less). Such a sequence is said to have

65% sequence identity to the listed sequence. In one embodiment, a substantially equivalent, *e.g.*, mutant, sequence of the invention varies from a listed sequence by no more than 30% (70% sequence identity); in a variation of this embodiment, by no more than 25% (75% sequence identity); and in a further variation of this embodiment, by no more than 20% (80% sequence identity) and in a further variation of this embodiment, by no more than 10% (90% sequence identity) and in a further variation of this embodiment, by no more that 5% (95% sequence identity). Substantially equivalent, *e.g.*, mutant, amino acid sequences according to the invention preferably have at least 80% sequence identity with a listed amino acid sequence, more preferably at least 90% sequence identity. Substantially equivalent nucleotide sequence of the invention can have lower percent sequence identities, taking into account, for example, the redundancy or degeneracy of the genetic code. Preferably, nucleotide sequence has at least about 65% identity, more preferably at least about 75% identity, and most preferably at least about 95% identity. For the purposes of the present invention, sequences having substantially equivalent biological activity and substantially equivalent expression characteristics are considered substantially equivalent. For the purposes of determining equivalence, truncation of the mature sequence (*e.g.*, via a mutation which creates a spurious stop codon) should be disregarded. Sequence identity may be determined, e.g., using the Jotun Hein method (Hein, J. (1990) Methods Enzymol. 183:626-645). Identity between sequences can also be determined by other methods known in the art, e.g. by varying hybridization conditions.

The term "totipotent" refers to the capability of a cell to differentiate into all of the cell types of an adult organism.

The term "transformation" means introducing DNA into a suitable host cell so that the DNA is replicable, either as an extrachromosomal element, or by chromosomal integration. The term "transfection" refers to the taking up of an expression vector by a suitable host cell, whether or not any coding sequences are in fact expressed. The term "infection" refers to the introduction of nucleic acids into a suitable host cell by use of a virus or viral vector.

As used herein, an "uptake modulating fragment," UMF, means a series of nucleotides which mediate the uptake of a linked DNA fragment into a cell. UMFs can

be readily identified using known UMFs as a target sequence or target motif with the computer-based systems described below. The presence and activity of a UMF can be confirmed by attaching the suspected UMF to a marker sequence. The resulting nucleic acid molecule is then incubated with an appropriate host under appropriate conditions and the uptake of the marker sequence is determined. As described above, a UMF will increase the frequency of uptake of a linked marker sequence.

Each of the above terms is meant to encompass all that is described for each, unless the context dictates otherwise.

4.2 NUCLEIC ACIDS OF THE INVENTION

The invention is based on the discovery of novel JPL polypeptides, the polynucleotides encoding the JPL polypeptides and the use of these compositions for the diagnosis, treatment or prevention of cancers and other immunological disorders.

The isolated polynucleotides of the invention include, but are not limited to a polynucleotide comprising any of the nucleotide sequences of SEQ ID NO: 1, 2, or 3; a fragment of SEQ ID NO: 1, 2, or 3; a polynucleotide comprising the full length protein coding sequence of SEQ ID NO: 1, 2, or 3 (for example coding for SEQ ID NO: 4 or 5). The polynucleotides of the present invention also include, but are not limited to, a polynucleotide that hybridizes under stringent conditions to (a) the complement of any of the nucleotides sequences of SEQ ID NO: 1, 2 or 3; (b) a polynucleotide encoding any one of the polypeptides of SEQ ID NO: 4 or 5; (c) a polynucleotide which is an allelic variant of any polynucleotides recited above; (d) a polynucleotide which encodes a species homolog of any of the proteins recited above; or (e) a polynucleotide that encodes a polypeptide comprising a specific domain or truncation of the polypeptides of SEQ ID NO: 4 or 5. Domains of interest may depend on the nature of the encoded polypeptide; e.g., domains in receptor-like polypeptides include ligand-binding, extracellular, transmembrane, or cytoplasmic domains, or combinations thereof; domains in immunoglobulin-like proteins include the variable immunoglobulin-like domains; domains in enzyme-like polypeptides include catalytic and substrate binding domains; and domains in ligand polypeptides include receptor-binding domains.

The polynucleotides of the invention include naturally occurring or wholly or partially synthetic DNA, e.g., cDNA and genomic DNA, and RNA, e.g., mRNA. The polynucleotides may include all of the coding region of the cDNA or may represent a portion of the coding region of the cDNA.

The present invention also provides genes corresponding to the cDNA sequences disclosed herein. The corresponding genes can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include the preparation of probes or primers from the disclosed sequence information for identification and/or amplification of genes in appropriate genomic libraries or other sources of genomic materials. Further 5' and 3' sequence can be obtained using methods known in the art. For example, full length cDNA or genomic DNA that corresponds to any of the polynucleotides of SEQ ID NO: 1, 2, or 3 can be obtained by screening appropriate cDNA or genomic DNA libraries under suitable hybridization conditions using any of the polynucleotides of SEQ ID NO: 1, 2, or 3 or a portion thereof as a probe. Alternatively, the polynucleotides of SEQ ID NO: 1, 2, or 3 may be used as the basis for suitable primer(s) that allow identification and/or amplification of genes in appropriate genomic DNA or cDNA libraries.

The nucleic acid sequences of the invention can be assembled from ESTs and sequences (including cDNA and genomic sequences) obtained from one or more public databases, such as dbEST, gbpri, and UniGene. The EST sequences can provide identifying sequence information, representative fragment or segment information, or novel segment information for the full-length gene.

The polynucleotides of the invention also provide polynucleotides including nucleotide sequences that are substantially equivalent to the polynucleotides recited above. Polynucleotides according to the invention can have, *e.g.*, at least about 65%, at least about 70%, at least about 75%, at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, or 89%, more typically at least about 90%, 91%, 92%, 93%, or 94% and even more typically at least about 95%, 96%, 97%, 98% or 99% sequence identity to a polynucleotide recited above.

Included within the scope of the nucleic acid sequences of the invention are nucleic acid sequence fragments that hybridize under stringent conditions to any of the

nucleotide sequences of SEQ ID NO: 1, 2, or 3 or complements thereof, which fragment is greater than about 5 nucleotides, preferably 7 nucleotides, more preferably greater than 9 nucleotides and most preferably greater than 17 nucleotides. Fragments of, e.g. 15, 17, or 20 nucleotides or more that are selective for (i.e. specifically hybridize to any one of the polynucleotides of the invention) are contemplated. Probes capable of specifically hybridizing to a polynucleotide can differentiate polynucleotide sequences of the invention from other polynucleotide sequences in the same family of genes or can differentiate human genes from genes of other species, and are preferably based on unique nucleotide sequences.

The sequences falling within the scope of the present invention are not limited to these specific sequences, but also include allelic and species variations thereof. Allelic and species variations can be routinely determined by comparing the sequence provided in SEQ ID NO: 1, 2, or 3 a representative fragment thereof, or a nucleotide sequence at least 90% identical, preferably 95% identical, to SEQ ID NO: 1, 2, or 3 with a sequence from another isolate of the same species. Furthermore, to accommodate codon variability, the invention includes nucleic acid molecules coding for the same amino acid sequences as do the specific ORFs disclosed herein. In other words, in the coding region of an ORF, substitution of one codon for another codon that encodes the same amino acid is expressly contemplated.

The nearest neighbor result for the nucleic acids of the present invention, including SEQ ID NO: 1, 2, or 3, can be obtained by searching a database using an algorithm or a program. Preferably, a BLAST which stands for Basic Local Alignment Search Tool is used to search for local sequence alignments (Altshul, S.F. J Mol. Evol. 36 290-300 (1993) and Altschul S.F. et al. J. Mol. Biol. 21:403-410 (1990))

Species homologs (or orthologs) of the disclosed polynucleotides and proteins are also provided by the present invention. Species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source from the desired species.

The invention also encompasses allelic variants of the disclosed polynucleotides or proteins; that is, naturally-occurring alternative forms of the isolated polynucleotide

which also encode proteins which are identical, homologous or related to that encoded by the polynucleotides.

The nucleic acid sequences of the invention are further directed to sequences which encode variants of the described nucleic acids. These amino acid sequence variants may be prepared by methods known in the art by introducing appropriate nucleotide changes into a native or variant polynucleotide. There are two variables in the construction of amino acid sequence variants: the location of the mutation and the nature of the mutation. Nucleic acids encoding the amino acid sequence variants are preferably constructed by mutating the polynucleotide to encode an amino acid sequence that does not occur in nature. These nucleic acid alterations can be made at sites that differ in the nucleic acids from different species (variable positions) or in highly conserved regions (constant regions). Sites at such locations will typically be modified in series, *e.g.*, by substituting first with conservative choices (*e.g.*, hydrophobic amino acid to a different hydrophobic amino acid) and then with more distant choices (*e.g.*, hydrophobic amino acid to a charged amino acid), and then deletions or insertions may be made at the target site. Amino acid sequence deletions generally range from about 1 to 30 residues, preferably about 1 to 10 residues, and are typically contiguous. Amino acid insertions include amino- and/or carboxyl-terminal fusions ranging in length from one to one hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Intrasequence insertions may range generally from about 1 to 10 amino residues, preferably from 1 to 5 residues. Examples of terminal insertions include the heterologous signal sequences necessary for secretion or for intracellular targeting in different host cells and sequences such as FLAG or poly-histidine sequences useful for purifying the expressed protein.

In a preferred method, polynucleotides encoding the novel amino acid sequences are changed via site-directed mutagenesis. This method uses oligonucleotide sequences to alter a polynucleotide to encode the desired amino acid variant, as well as sufficient adjacent nucleotides on both sides of the changed amino acid to form a stable duplex on either side of the site being changed. In general, the techniques of site-directed mutagenesis are well known to those of skill in the art and this technique is exemplified by publications such as, Edelman et al., *DNA* 2:183 (1983). A versatile and efficient

method for producing site-specific changes in a polynucleotide sequence was published by Zoller and Smith, *Nucleic Acids Res.* 10:6487-6500 (1982). PCR may also be used to create amino acid sequence variants of the novel nucleic acids. When small amounts of template DNA are used as starting material, primer(s) that differs slightly in sequence from the corresponding region in the template DNA can generate the desired amino acid variant. PCR amplification results in a population of product DNA fragments that differ from the polynucleotide template encoding the polypeptide at the position specified by the primer. The product DNA fragments replace the corresponding region in the plasmid and this gives a polynucleotide encoding the desired amino acid variant.

A further technique for generating amino acid variants is the cassette mutagenesis technique described in Wells et al., *Gene* 34:315 (1985); and other mutagenesis techniques well known in the art, such as, for example, the techniques in Sambrook et al., supra, and *Current Protocols in Molecular Biology*, Ausubel et al. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be used in the practice of the invention for the cloning and expression of these novel nucleic acids. Such DNA sequences include those which are capable of hybridizing to the appropriate novel nucleic acid sequence under stringent conditions.

Polynucleotides encoding preferred polypeptide truncations of the invention can be used to generate polynucleotides encoding chimeric or fusion proteins comprising one or more domains of the invention and heterologous protein sequences.

The polynucleotides of the invention additionally include the complement of any of the polynucleotides recited above. The polynucleotide can be DNA (genomic, cDNA, amplified, or synthetic) or RNA. Methods and algorithms for obtaining such polynucleotides are well known to those of skill in the art and can include, for example, methods for determining hybridization conditions that can routinely isolate polynucleotides of the desired sequence identities.

A polynucleotide according to the invention can be joined to any of a variety of other nucleotide sequences by well-established recombinant DNA techniques (see Sambrook J et al. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, NY). Useful nucleotide sequences for joining to polynucleotides include an

assortment of vectors, e.g., plasmids, cosmids, lambda phage derivatives, phagemids, and the like, that are well known in the art. Accordingly, the invention also provides a vector including a polynucleotide of the invention and a host cell containing the polynucleotide. In general, the vector contains an origin of replication functional in at least one organism, convenient restriction endonuclease sites, and a selectable marker for the host cell. Vectors according to the invention include expression vectors, replication vectors, probe generation vectors, and sequencing vectors. A host cell according to the invention can be a prokaryotic or eukaryotic cell and can be a unicellular organism or part of a multicellular organism.

The present invention further provides recombinant constructs comprising a nucleic acid having any of the nucleotide sequences of SEQ ID NO: 1, 2, or 3 or a fragment thereof or any other polynucleotides of the invention. In one embodiment, the recombinant constructs of the present invention comprise a vector, such as a plasmid or viral vector, into which a nucleic acid having any of the nucleotide sequences of SEQ ID NO: 1, 2, or 3 or a fragment thereof is inserted, in a forward or reverse orientation. In the case of a vector comprising one of the ORFs of the present invention, the vector may further comprise regulatory sequences, including for example, a promoter, operably linked to the ORF. Large numbers of suitable vectors and promoters are known to those of skill in the art and are commercially available for generating the recombinant constructs of the present invention. The following vectors are provided by way of example. Bacterial: pBs, phagescript, PsiX174, pBluescript SK, pBs KS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene); pTrc99A, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pWLneo, pSV2cat, pOG44, PXTI, pSG (Stratagene) pSVK3, pBPV, pMSG, and pSVL (Pharmacia).

The isolated polynucleotide of the invention may be operably linked to an expression control sequence such as the pMT2 or pED expression vectors disclosed in Kaufman et al., *Nucleic Acids Res.* 19, 4485-4490 (1991), in order to produce the protein recombinantly. Many suitable expression control sequences are known in the art. General methods of expressing recombinant proteins are also known and are exemplified in R. Kaufman, *Methods in Enzymology* 185, 537-566 (1990). As defined herein "operably linked" means that the isolated polynucleotide of the invention and an

expression control sequence are situated within a vector or cell in such a way that the protein is expressed by a host cell which has been transformed (transfected) with the ligated polynucleotide/expression control sequence.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda PR, and trc. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art. Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, *e.g.*, the ampicillin resistance gene of *E. coli* and *S. cerevisiae* TRP1 gene, and a promoter derived from a highly expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), a-factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an amino terminal identification peptide imparting desired characteristics, *e.g.*, stabilization or simplified purification of expressed recombinant product. Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium* and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*, although others may also be employed as a matter of choice.

As a representative but non-limiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM 1 (Promega Biotech, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed. Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced or derepressed by appropriate means (*e.g.*, temperature shift or chemical induction) and cells are cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Polynucleotides of the invention can also be used to induce immune responses. For example, as described in Fan et al., *Nat. Biotech.* 17:870-872 (1999), incorporated herein by reference, nucleic acid sequences encoding a polypeptide may be used to generate antibodies against the encoded polypeptide following topical administration of naked plasmid DNA or following injection, and preferably intramuscular injection of the DNA. The nucleic acid sequences are preferably inserted in a recombinant expression vector and may be in the form of naked DNA.

4.2.1 ANTISENSE NUCLEIC ACIDS

Another aspect of the invention pertains to isolated antisense nucleic acid molecules that can hybridize to, or are complementary to, the nucleic acid molecule comprising the JPL nucleotide sequences, or fragments, analogs or derivatives thereof. An "antisense" nucleic acid comprises a nucleotide sequence that is complementary to a "sense" nucleic acid encoding a protein (e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence). In specific aspects, antisense nucleic acid molecules are provided that comprise a sequence complementary to at least about 10, 25, 50, 100, 250 or 500 nucleotides or an entire JPL coding strand, or to only a portion thereof. Nucleic acid molecules encoding fragments,

homologs, derivatives and analogs of a JPL-like or antisense nucleic acids complementary to a JPL nucleic acid sequence of are additionally provided.

In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding a JPL protein. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues. In another embodiment, the antisense nucleic acid molecule is antisense to a "conceding region" of the coding strand of a nucleotide sequence encoding the JPL protein. The term "conceding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (i.e., also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding the JPL protein disclosed herein, antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick or Hoogsteen base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of JPL mRNA, but more preferably is an oligonucleotide that is antisense to only a portion of the coding or noncoding region of JPL mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of JPL polypeptide mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis or enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids (e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used).

Examples of modified nucleotides that can be used to generate the antisense nucleic acid include: 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-

methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following section).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated in situ such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a JPL protein to thereby inhibit expression of the protein (e.g., by inhibiting transcription and/or translation). The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule that binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention includes direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface (e.g., by linking the antisense nucleic acid molecules to peptides or antibodies that bind to cell surface receptors or antigens). The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient nucleic acid molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an alpha-anomeric nucleic acid molecule. An alpha-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to

the usual alpha-units, the strands run parallel to each other. See, e.g., Gaultier, et al., 1987. Nucl. Acids Res. 15: 6625-6641. The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (see, e.g., Inoue, et al. 1987. Nucl. Acids Res. 15: 6131-6148) or a chimeric RNA-DNA analogue (see, e.g., Inoue, et al., 1987. FEBS Lett. 215: 327-330.

4.2.2 RIBOZYMES AND PNA MOIETIES

Nucleic acid modifications include, by way of non-limiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they can be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject.

In one embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (e.g., hammerhead ribozymes as described in Haselhoff and Gerlach 1988. Nature 334: 585-591) can be used to catalytically cleave JPL mRNA transcripts to thereby inhibit translation of JPL mRNA. A ribozyme having specificity for a JPL-encoding nucleic acid can be designed based upon the nucleotide sequence of a JPL cDNA disclosed herein. For example, a derivative of a Tetrahymena L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a JPL-encoding mRNA. See, e.g., U.S. Patent 4,987,071 to Cech, et al. and U.S. Patent 5,116,742 to Cech, et al. Stem cell growth factor-like mRNA can also be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel et al., (1993) Science 261:1411-1418.

Alternatively, JPL gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the JPL nucleic acid (e.g., the JPL promoter and/or enhancers) to form triple helical structures that prevent transcription of the JPL gene in target cells. See, e.g., Helene, 1991. Anticancer Drug Des. 6: 569-84;

Helene, et al. 1992. Ann. N.Y. Acad. Sci. 660: 27-36; Maher, 1992. Bioassays 14: 807-15.

In various embodiments, the JPL nucleic acids can be modified at the base moiety, sugar moiety or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids. See, e.g., Hyrup, et al., 1996. Bioorg Med Chem 4: 5-23. As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics (e.g., DNA mimics) in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup, et al., 1996, supra; Perry-O'Keefe, et al., (Proc. Natl. Acad. Sci. USA 93: 14670-14675 (1996)).

PNAs of JPL can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, e.g., inducing transcription or translation arrest or inhibiting replication. PNAs of JPL can also be used, for example, in the analysis of single base pair mutations in a gene (e.g., PNA directed PCR clamping; as artificial restriction enzymes when used in combination with other enzymes, e.g., S1 nucleases (see, Hyrup, et al., 1996, supra); or as probes or primers for DNA sequence and hybridization (see, Hyrup, et al., 1996, supra; Perry-O'Keefe, et al., 1996, supra).

In another embodiment, PNAs of JPL can be modified, e.g., to enhance their stability or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of JPL can be generated that may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes (e.g., RNase H and DNA polymerases) to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (see, Hyrup,

et al., 1996, supra). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup, et al., 1996. Supra, et al., 1996. Nucl Acids Res 24: 3357-3363. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry, and modified nucleoside analogs, e.g., 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used between the PNA and the 5' end of DNA. See, e.g., Mag, et al., 1989. Nucl Acid Res 17: 5973-5988. PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment. See, e.g., Finn, et al., 1996. supra. Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment. See, e.g., Petersen, et al., 1975. Bioorg. Med. Chem. Lett. 5: 1119-11124.

In other embodiments, the oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors in vivo), or agents facilitating transport across the cell membrane (see, e.g., Letsinger, et al., 1989. Proc. Natl. Acad. Sci. U.S.A. 86: 6553-6556; Lemaitre, et al., 1987. Proc. Natl. Acad. Sci. 84: 648-652; PCT Publication No. WO88/09810) or the blood-brain barrier (see, e.g., PCT Publication No. WO 89/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (see, e.g., Krol, et al., 1988. BioTechniques 6:958-976) or intercalating agents (see, e.g., Zon, 1988. Pharm. Res. 5: 539-549). To this end, the oligonucleotide can be conjugated to another molecule, e.g., a peptide, a hybridization triggered cross-linking agent, a transport agent, a hybridization-triggered cleavage agent, and the like.

4.3 HOSTS

The present invention further provides host cells genetically engineered to contain the polynucleotides of the invention. For example, such host cells may contain nucleic acids of the invention introduced into the host cell using known transformation, transfection or infection methods. The present invention still further provides host cells genetically engineered to express the polynucleotides of the invention, wherein such polynucleotides are in operative association with a regulatory sequence heterologous to the host cell which drives expression of the polynucleotides in the cell.

The host cell can be a higher eukaryotic host cell, such as a mammalian cell, a lower eukaryotic host cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the recombinant construct into the host cell can be effected by calcium phosphate transfection, DEAE, dextran mediated transfection, or electroporation (Davis, L. et al., Basic Methods in Molecular Biology (1986)). The host cells containing one of polynucleotides of the invention, can be used in conventional manners to produce the gene product encoded by the isolated fragment (in the case of an ORF) or can be used to produce a heterologous protein under the control of the EMF.

Any host/vector system can be used to express one or more of the ORFs of the present invention. These include, but are not limited to, eukaryotic hosts such as HeLa cells, Cv-1 cell, COS cells, and Sf9 cells, as well as prokaryotic host such as E. coli and B. subtilis. The most preferred cells are those which do not normally express the particular polypeptide or protein or which expresses the polypeptide or protein at low natural level. Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., in Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, New York (1989), the disclosure of which is hereby incorporated by reference.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell tines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early promoter, enhancer, splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements. Recombinant polypeptides and proteins produced in bacterial culture are

usually isolated by initial extraction from cell pellets, followed by one or more salting-out, aqueous ion exchange or size exclusion chromatography steps. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

A number of types of cells may act as suitable host cells for expression of the protein. Mammalian host cells include, for example, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK or Jurkat cells.

Alternatively, it may be possible to produce the protein in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Potentially suitable yeast strains include Saccharomyces cerevisiae, Schizosaccharomyces pombe, Kluyveromyces strains, Candida, or any yeast strain capable of expressing heterologous proteins. Potentially suitable bacterial strains include Escherichia coli, Bacillus subtilis, Salmonella typhimurium, or any bacterial strain capable of expressing heterologous proteins. If the protein is made in yeast or bacteria, it may be necessary to modify the protein produced therein, for example by phosphorylation or glycosylation of the appropriate sites, in order to obtain the functional protein. Such covalent attachments may be accomplished using known chemical or enzymatic methods.

In another embodiment of the present invention, cells and tissues may be engineered to express an endogenous gene comprising the polynucleotides of the invention under the control of inducible regulatory elements, in which case the regulatory sequences of the endogenous gene may be replaced by homologous recombination. As described herein, gene targeting can be used to replace a gene's existing regulatory region with a regulatory sequence isolated from a different gene or a novel regulatory sequence synthesized by genetic engineering methods. Such regulatory sequences may be comprised of promoters, enhancers, scaffold-attachment regions, negative regulatory

elements, transcriptional initiation sites, regulatory protein binding sites or combinations of said sequences. Alternatively, sequences which affect the structure or stability of the RNA or protein produced may be replaced, removed, added, or otherwise modified by targeting, including polyadenylation signals, mRNA stability elements, splice sites, leader sequences for enhancing or modifying transport or secretion properties of the protein, or other sequences which alter or improve the function or stability of protein or RNA molecules.

The targeting event may be a simple insertion of the regulatory sequence, placing the gene under the control of the new regulatory sequence, e.g., inserting a new promoter or enhancer or both upstream of a gene. Alternatively, the targeting event may be a simple deletion of a regulatory element, such as the deletion of a tissue-specific negative regulatory element. Alternatively, the targeting event may replace an existing element; for example, a tissue-specific enhancer can be replaced by an enhancer that has broader or different cell-type specificity than the naturally occurring elements. Here, the naturally occurring sequences are deleted and new sequences are added. In all cases, the identification of the targeting event may be facilitated by the use of one or more selectable marker genes that are contiguous with the targeting DNA, allowing for the selection of cells in which the exogenous DNA has integrated into the host cell genome. The identification of the targeting event may also be facilitated by the use of one or more marker genes exhibiting the property of negative selection, such that the negatively selectable marker is linked to the exogenous DNA, but configured such that the negatively selectable marker flanks the targeting sequence, and such that a correct homologous recombination event with sequences in the host cell genome does not result in the stable integration of the negatively selectable marker. Markers useful for this purpose include the Herpes Simplex Virus thymidine kinase (TK) gene or the bacterial xanthine-guanine phosphoribosyl-transferase (gpt) gene.

The gene targeting or gene activation techniques which can be used in accordance with this aspect of the invention are more particularly described in U.S. Patent No. 5,272,071 to Chappel; U.S. Patent No. 5,578,461 to Sherwin et al.; International Application No. PCT/US92/09627 (WO93/09222) by Selden et al.; and International

Application No. PCT/US90/06436 (WO91/06667) by Skoultchi et al., each of which is incorporated by reference herein in its entirety.

4.3.1 CHIMERIC AND FUSION PROTEINS

The invention also provides JPL chimeric or fusion proteins. As used herein, a JPL "chimeric protein" or "fusion protein" comprises a JPL polypeptide operatively linked to a non-JPL polypeptide. A "JPL polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a JPL protein, whereas a "non-JPL polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein that is not substantially homologous to the JPL protein, e.g., a protein that is different from the JPL protein and that is derived from the same or a different organism. Within a JPL fusion protein the JPL polypeptide can correspond to all or a portion of a JPL protein. In one embodiment, a JPL fusion protein comprises at least one biologically active portion of a JPL protein. In another embodiment, a JPL fusion protein comprises at least two biologically active portions of a JPL protein. In yet another embodiment, a JPL fusion protein comprises at least three biologically active portions of a JPL protein. Within the fusion protein, the term "operatively-linked" is intended to indicate that the JPL polypeptide and the non-JPL polypeptide are fused in-frame with one another. The non-JPL polypeptide can be fused to the N-terminus or C-terminus of the JPL polypeptide.

In one embodiment, the fusion protein is a GST- JPL fusion protein in which the JPL sequences are fused to the C-terminus of the GST (glutathione S-transferase) sequences. Such fusion proteins can facilitate the purification of recombinant JPL polypeptides. In another embodiment, the fusion protein is a JPL protein containing a heterologous signal sequence at its N-terminus. In certain host cells (e.g., mammalian host cells), expression and/or secretion of JPL can be increased through use of a heterologous signal sequence.

In yet another embodiment, the fusion protein is a JPL-immunoglobulin fusion protein in which the JPL sequences are fused to sequences derived from a member of the immunoglobulin protein family. The JPL-immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between a JPL ligand and a JPL protein on the surface of

a cell, to thereby suppress JPL-mediated signal transduction in vivo. The JPL-immunoglobulin fusion proteins can be used to affect the bioavailability of a JPL cognate ligand. Inhibition of the JPL ligand/JPL interaction can be useful therapeutically for both the treatment of proliferative and differentiative disorders, as well as modulating (e.g. promoting or inhibiting) cell survival. Moreover, the JPL-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-JPL antibodies in a subject, to purify JPL ligands, and in screening assays to identify molecules that inhibit the interaction of JPL with a JPL ligand.

A JPL chimeric or fusion protein of the invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, e.g., by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, e.g., Ausubel, et al. (eds.) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (e.g., a GST polypeptide). A JPL-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the JPL protein.

4.4 POLYPEPTIDES OF THE INVENTION

The isolated polypeptides of the invention include, but are not limited to, a polypeptide comprising: the amino acid sequence set forth as any one of SEQ ID NO: 4 or 5 or an amino acid sequence encoded by any one of the nucleotide sequences SEQ ID NO: 1, 2, or 3 or the corresponding full length. Polypeptides of the invention also include polypeptides preferably with biological or immunological activity that are encoded by:

(a) a polynucleotide having any one of the nucleotide sequences set forth in SEQ ID NO: 1, 2, or 3 or (b) polynucleotides encoding any one of the amino acid sequences set forth as SEQ ID NO: 4 or 5 or (c) polynucleotides that hybridize to the complement of the polynucleotides of either (a) or (b) under stringent hybridization conditions. The invention also provides biologically active or immunologically active variants of any of the amino acid sequences set forth as SEQ ID NO: 4 or 5 or the corresponding full length protein; and "substantial equivalents" thereof (e.g., with at least about 65%, at least about 70%, at least about 75%, at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, or 89%, more typically at least about 90%, 91%, 92%, 93%, or 94% and even more typically at least about 95%, 96%, 97%, 98% or 99%, most typically at least about 99% amino acid identity) that retain biological activity. Polypeptides encoded by allelic variants may have a similar, increased, or decreased activity compared to polypeptides comprising SEQ ID NO: 4 or 5.

Fragments of the proteins of the present invention which are capable of exhibiting biological activity are also encompassed by the present invention. Fragments of the protein may be in linear form or they may be cyclized using known methods, for example, as described in H. U. Saragovi, et al., Bio/Technology 10, 773-778 (1992) and in R. S. McDowell, et al., J. Amer. Chem. Soc. 114, 9245-9253 (1992), both of which are incorporated herein by reference. Such fragments may be fused to carrier molecules such as immunoglobulins for many purposes, including increasing the valency of protein binding sites.

Protein compositions of the present invention may further comprise an acceptable carrier, such as a hydrophilic, *e.g.*, pharmaceutically acceptable, carrier.

The present invention further provides isolated polypeptides encoded by the nucleic acid fragments of the present invention or by degenerate variants of the nucleic acid fragments of the present invention. By "degenerate variant" is intended nucleotide fragments which differ from a nucleic acid fragment of the present invention (*e.g.*, an ORF) by nucleotide sequence but, due to the degeneracy of the genetic code, encode an identical polypeptide sequence. Preferred nucleic acid fragments of the present invention are the ORFs that encode proteins.

A variety of methodologies known in the art can be utilized to obtain any one of the isolated polypeptides or proteins of the present invention. At the simplest level, the amino acid sequence can be synthesized using commercially available peptide synthesizers. The synthetically-constructed protein sequences, by virtue of sharing primary, secondary or tertiary structural and/or conformational characteristics with proteins may possess biological properties in common therewith, including protein activity. This technique is particularly useful in producing small peptides and fragments of larger polypeptides. Fragments are useful, for example, in generating antibodies against the native polypeptide. Thus, they may be employed as biologically active or immunological substitutes for natural, purified proteins in screening of therapeutic compounds and in immunological processes for the development of antibodies.

The polypeptides and proteins of the present invention can alternatively be purified from cells which have been altered to express the desired polypeptide or protein. As used herein, a cell is said to be altered to express a desired polypeptide or protein when the cell, through genetic manipulation, is made to produce a polypeptide or protein which it normally does not produce or which the cell normally produces at a lower level. One skilled in the art can readily adapt procedures for introducing and expressing either recombinant or synthetic sequences into eukaryotic or prokaryotic cells in order to generate a cell which produces one of the polypeptides or proteins of the present invention.

The invention also relates to methods for producing a polypeptide comprising growing a culture of host cells of the invention in a suitable culture medium, and purifying the protein from the cells or the culture in which the cells are grown. For example, the methods of the invention include a process for producing a polypeptide in which a host cell containing a suitable expression vector that includes a polynucleotide of the invention is cultured under conditions that allow expression of the encoded polypeptide. The polypeptide can be recovered from the culture, conveniently from the culture medium, or from a lysate prepared from the host cells and further purified. Preferred embodiments include those in which the protein produced by such process is a full length or mature form of the protein.

In an alternative method, the polypeptide or protein is purified from bacterial cells which naturally produce the polypeptide or protein. One skilled in the art can readily follow known methods for isolating polypeptides and proteins in order to obtain one of the isolated polypeptides or proteins of the present invention. These include, but are not limited to, immunochromatography, HPLC, size-exclusion chromatography, ion-exchange chromatography, and immuno-affinity chromatography. See, *e.g.*, Scopes, *Protein Purification: Principles and Practice*, Springer-Verlag (1994); Sambrook, et al., in Molecular Cloning: *A Laboratory Manual*; Ausubel et al., *Current Protocols in Molecular Biology*. Polypeptide fragments that retain biological/immunological activity include fragments comprising greater than about 100 amino acids, or greater than about 200 amino acids, and fragments that encode specific protein domains.

The purified polypeptides can be used in *in vitro* binding assays which are well known in the art to identify molecules which bind to the polypeptides. These molecules include but are not limited to, for e.g., small molecules, molecules from combinatorial libraries, antibodies or other proteins. The molecules identified in the binding assay are then tested for antagonist or agonist activity in *in vivo* tissue culture or animal models that are well known in the art. In brief, the molecules are titrated into a plurality of cell cultures or animals and then tested for either cell/animal death or prolonged survival of the animal/cells.

In addition, the peptides of the invention or molecules capable of binding to the peptides may be complexed with toxins, e.g., ricin or cholera, or with other compounds that are toxic to cells. The toxin-binding molecule complex is then targeted to a tumor or other cell by the specificity of the binding molecule for SEQ ID NO: 4 or 5.

The protein of the invention may also be expressed as a product of transgenic animals, *e.g.,* as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a nucleotide sequence encoding the protein.

The proteins provided herein also include proteins characterized by amino acid sequences similar to those of purified proteins but into which modification are naturally provided or deliberately engineered. For example, modifications, in the peptide or DNA sequence, can be made by those skilled in the art using known techniques. Modifications

of interest in the protein sequences may include the alteration, substitution, replacement, insertion or deletion of a selected amino acid residue in the coding sequence. For example, one or more of the cysteine residues may be deleted or replaced with another amino acid to alter the conformation of the molecule. Techniques for such alteration, substitution, replacement, insertion or deletion are well known to those skilled in the art (see, *e.g.*, U.S. Pat. No. 4,518,584). Preferably, such alteration, substitution, replacement, insertion or deletion retains the desired activity of the protein. Regions of the protein that are important for the protein function can be determined by various methods known in the art including the alanine-scanning method which involved systematic substitution of single or strings of amino acids with alanine, followed by testing the resulting alanine-containing variant for biological activity. This type of analysis determines the importance of the substituted amino acid(s) in biological activity. Regions of the protein that are important for protein function may be determined by the eMATRIX program.

Other fragments and derivatives of the sequences of proteins which would be expected to retain protein activity in whole or in part and are useful for screening or other immunological methodologies may also be easily made by those skilled in the art given the disclosures herein. Such modifications are encompassed by the present invention.

The protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *e.g.*, Invitrogen, San Diego, Calif., U.S.A. (the MaxBat™ kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference. As used herein, an insect cell capable of expressing a polynucleotide of the present invention is "transformed."

The protein of the invention may be prepared by culturing transformed host cells under culture conditions suitable to express the recombinant protein. The resulting expressed protein may then be purified from such culture (*i.e.*, from culture medium or cell extracts) using known purification processes, such as gel filtration and ion exchange chromatography. The purification of the protein may also include an affinity column

containing agents which will bind to the protein; one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl™ or Cibacrom blue 3GA Sepharose™; one or more steps involving hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or immunoaffinity chromatography.

Alternatively, the protein of the invention may also be expressed in a form which will facilitate purification. For example, it may be expressed as a fusion protein, such as those of maltose binding protein (MBP), glutathione-S-transferase (GST) or thioredoxin (TRX), or as a His tag. Kits for expression and purification of such fusion proteins are commercially available from New England BioLab (Beverly, Mass.), Pharmacia (Piscataway, N.J.) and Invitrogen, respectively. The protein can also be tagged with an epitope and subsequently purified by using a specific antibody directed to such epitope. One such epitope ("FLAG®") is commercially available from Kodak (New Haven, Conn.).

Finally, one or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, *e.g.*, silica gel having pendant methyl or other aliphatic groups, can be employed to further purify the protein. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a substantially homogeneous isolated recombinant protein. The protein thus purified is substantially free of other mammalian proteins and is defined in accordance with the present invention as an "isolated protein."

The polypeptides of the invention include analogs (variants). The polypeptides of the invention include JPL analogs. This embraces fragments of JPL polypeptide of the invention, as well JPL polypeptides which comprise one or more amino acids deleted, inserted, or substituted. Also, analogs of the JPL polypeptides of the invention embrace fusions of the JPL polypeptides or modifications of the JPL polypeptides, wherein the JPL polypeptide or analog is fused to another moiety or moieties, e.g., targeting moiety or another therapeutic agent. Such analogs may exhibit improved properties such as activity and/or stability. Examples of moieties which may be fused to the JPL polypeptide or an analog include, for example, targeting moieties which provide for the delivery of polypeptide to neurons, e.g., antibodies to central nervous system, or antibodies to

receptor and ligands expressed on neuronal cells. Other moieties which may be fused to JPL polypeptides include therapeutic agents which are used for treatment, for example anti-depressant drugs or other medications for neurological disorders. Also, JPL polypeptides may be fused to neuron growth modulators, and other chemokines for targeted delivery.

4.4.1 DETERMINING POLYPEPTIDE AND POLYNUCLEOTIDE IDENTITY AND SIMILARITY

Preferred identity and/or similarity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in computer programs including, but are not limited to, the GCG program package, including GAP (Devereux, J., et al., Nucleic Acids Research 12(1):387 (1984); Genetics Computer Group, University of Wisconsin, Madison, WI), BLASTP, BLASTN, BLASTX, FASTA (Altschul, S.F. et al., J. Molec. Biol. 215:403-410 (1990), PSI-BLAST (Altschul S.F. et al., Nucleic Acids Res. vol. 25, pp. 3389-3402, herein incorporated by reference), the eMatrix software (Wu et al., J. Comp. Biol., vol. 6, pp. 219-235 (1999), herein incorporated by reference), eMotif software (Nevill-Manning et al, ISMB-97, vol 4, pp. 202-209, herein incorporated by reference), the GeneAtlas software (Molecular Simulations Inc. (MSI), San Diego, CA) (Sanchez and Sali (1998) Proc. Natl. Acad. Sci., 95, 13597-13602; Kitson DH et al, (2000) "Remote homology detection using structural modeling – an evaluation" Submitted; Fischer and Eisenberg (1996) Protein Sci. 5, 947-955), and the Kyte-Doolittle hydrophobocity prediction algorithm (J. Mol Biol, 157, pp. 105-31 (1982), incorporated herein by reference). The BLAST programs are publicly available from the National Center for Biotechnology Information (NCBI) and other sources (BLAST Manual, Altschul, S., et al. NCB NLM NIH Bethesda, MD 20894; Altschul, S., et al., J. Mol. Biol. 215:403-410 (1990).

4.5 GENE THERAPY

Mutations in the gene of the polynucleotides of the invention may result in loss of normal function of the encoded protein. The invention thus provides gene therapy to

restore normal activity of the polypeptides of the invention; or to treat disease states involving polypeptides of the invention. Delivery of a functional gene encoding polypeptides of the invention to appropriate cells is effected *ex vivo*, *in situ*, or *in vivo* by use of vectors, and more particularly viral vectors (e.g., adenovirus, adeno-associated virus, or a retrovirus), or *ex vivo* by use of physical DNA transfer methods (e.g., liposomes or chemical treatments). See, for example, Anderson, Nature, supplement to vol. 392, no. 6679, pp.25-20 (1998). For additional reviews of gene therapy technology see Friedmann, Science, 244: 1275-1281 (1989); Verma, Scientific American: 68-84 (1990); and Miller, Nature, 357: 455-460 (1992). Introduction of any one of the nucleotides of the present invention or a gene encoding the polypeptides of the present invention can also be accomplished with extrachromosomal substrates (transient expression) or artificial chromosomes (stable expression). Cells may also be cultured *ex vivo* in the presence of proteins of the present invention in order to proliferate or to produce a desired effect on or activity in such cells. Treated cells can then be introduced *in vivo* for therapeutic purposes. Alternatively, it is contemplated that in other human disease states, preventing the expression of or inhibiting the activity of polypeptides of the invention will be useful in treating the disease states. It is contemplated that antisense therapy or gene therapy could be applied to negatively regulate the expression of polypeptides of the invention.

Other methods inhibiting expression of a protein include the introduction of antisense molecules to the nucleic acids of the present invention, their complements, or their translated RNA sequences, by methods known in the art. Further, the polypeptides of the present invention can be inhibited by using targeted deletion methods, or the insertion of a negative regulatory element such as a silencer, which is tissue specific.

The present invention still further provides cells genetically engineered *in vivo* to express the polynucleotides of the invention, wherein such polynucleotides are in operative association with a regulatory sequence heterologous to the host cell which drives expression of the polynucleotides in the cell. These methods can be used to increase or decrease the expression of the polynucleotides of the present invention.

Knowledge of DNA sequences provided by the invention allows for modification of cells to permit, increase, or decrease, expression of endogenous polypeptide. Cells can

be modified (e.g., by homologous recombination) to provide increased polypeptide expression by replacing, in whole or in part, the naturally occurring promoter with all or part of a heterologous promoter so that the cells express the protein at higher levels. The heterologous promoter is inserted in such a manner that it is operatively linked to the desired protein encoding sequences. See, for example, PCT International Publication No. WO 94/12650, PCT International Publication No. WO 92/20808, and PCT International Publication No. WO 91/09955. It is also contemplated that, in addition to heterologous promoter DNA, amplifiable marker DNA (e.g., ada, dhfr, and the multifunctional CAD gene which encodes carbamyl phosphate synthase, aspartate transcarbamylase, and dihydroorotase) and/or intron DNA may be inserted along with the heterologous promoter DNA. If linked to the desired protein coding sequence, amplification of the marker DNA by standard selection methods results in co-amplification of the desired protein coding sequences in the cells.

In another embodiment of the present invention, cells and tissues may be engineered to express an endogenous gene comprising the polynucleotides of the invention under the control of inducible regulatory elements, in which case the regulatory sequences of the endogenous gene may be replaced by homologous recombination. As described herein, gene targeting can be used to replace a gene's existing regulatory region with a regulatory sequence isolated from a different gene or a novel regulatory sequence synthesized by genetic engineering methods. Such regulatory sequences may be comprised of promoters, enhancers, scaffold-attachment regions, negative regulatory elements, transcriptional initiation sites, regulatory protein binding sites or combinations of said sequences. Alternatively, sequences which affect the structure or stability of the RNA or protein produced may be replaced, removed, added, or otherwise modified by targeting. These sequences include polyadenylation signals, mRNA stability elements, splice sites, leader sequences for enhancing or modifying transport or secretion properties of the protein, or other sequences which alter or improve the function or stability of protein or RNA molecules.

The targeting event may be a simple insertion of the regulatory sequence, placing the gene under the control of the new regulatory sequence, *e.g.*, inserting a new promoter or enhancer or both upstream of a gene. Alternatively, the targeting event may be a

simple deletion of a regulatory element, such as the deletion of a tissue-specific negative regulatory element. Alternatively, the targeting event may replace an existing element; for example, a tissue-specific enhancer can be replaced by an enhancer that has broader or different cell-type specificity than the naturally occurring elements. Here, the naturally occurring sequences are deleted and new sequences are added. In all cases, the identification of the targeting event may be facilitated by the use of one or more selectable marker genes that are contiguous with the targeting DNA, allowing for the selection of cells in which the exogenous DNA has integrated into the cell genome. The identification of the targeting event may also be facilitated by the use of one or more marker genes exhibiting the property of negative selection, such that the negatively selectable marker is linked to the exogenous DNA, but configured such that the negatively selectable marker flanks the targeting sequence, and such that a correct homologous recombination event with sequences in the host cell genome does not result in the stable integration of the negatively selectable marker. Markers useful for this purpose include the Herpes Simplex Virus thymidine kinase (TK) gene or the bacterial xanthine-guanine phosphoribosyl-transferase (gpt) gene.

The gene targeting or gene activation techniques which can be used in accordance with this aspect of the invention are more particularly described in U.S. Patent No. 5,272,071 to Chappel; U.S. Patent No. 5,578,461 to Sherwin et al.; International Application No. PCT/US92/09627 (WO93/09222) by Selden et al.; and International Application No. PCT/US90/06436 (WO91/06667) by Skoultchi et al., each of which is incorporated by reference herein in its entirety.

4.6 TRANSGENIC ANIMALS

In preferred methods to determine biological functions of the polypeptides of the invention in vivo, one or more genes provided by the invention are either over expressed or inactivated in the germ line of animals using homologous recombination [Capecchi, Science 244:1288-1292 (1989)]. Animals in which the gene is over expressed, under the regulatory control of exogenous or endogenous promoter elements, are known as transgenic animals. Animals in which an endogenous gene has been inactivated by homologous recombination are referred to as "knockout" animals. Knockout animals,

preferably non-human mammals, can be prepared as described in U.S. Patent No. 5,557,032, incorporated herein by reference. Transgenic animals are useful to determine the roles polypeptides of the invention play in biological processes, and preferably in disease states. Transgenic animals are useful as model systems to identify compounds that modulate lipid metabolism. Transgenic animals, preferably non-human mammals, are produced using methods as described in U.S. Patent No 5,489,743 and PCT Publication No. WO94/28122, incorporated herein by reference.

Transgenic animals can be prepared wherein all or part of a promoter of the polynucleotides of the invention is either activated or inactivated to alter the level of expression of the polypeptides of the invention. Inactivation can be carried out using homologous recombination methods described above. Activation can be achieved by supplementing or even replacing the homologous promoter to provide for increased protein expression. The homologous promoter can be supplemented by insertion of one or more heterologous enhancer elements known to confer promoter activation in a particular tissue.

The polynucleotides of the present invention also make possible the development, through, e.g., homologous recombination or knock out strategies; of animals that fail to express functional JPL polypeptide or that express a variant of JPL polypeptide. Such animals are useful as models for studying the *in vivo* activities of JPL polypeptide as well as for studying modulators of the JPL polypeptide.

4.7 ANTIBODIES

Also included in the invention are antibodies to proteins, or fragments of proteins of the invention. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin (Ig) molecules, i.e., molecules that contain an antigen-binding site that specifically binds (immunoreacts with) an antigen. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F_{ab}, $F_{ab'}$ and $F_{(ab')2}$ fragments, and an F_{ab} expression library. In general, an antibody molecule obtained from humans relates to any of the classes IgG, IgM, IgA, IgE and IgD, which differ from one another by the nature of the heavy chain present in the molecule. Certain classes have subclasses as well, such as IgG_1, IgG_2, and

others. Furthermore, in humans, the light chain may be a kappa chain or a lambda chain. Reference herein to antibodies includes a reference to all such classes, subclasses and types of human antibody species.

An isolated related protein of the invention may be intended to serve as an antigen, or a portion or fragment thereof, and additionally can be used as an immunogen to generate antibodies that immunospecifically bind the antigen, using standard techniques for polyclonal and monoclonal antibody preparation. The full-length protein can be used or, alternatively, the invention provides antigenic peptide fragments of the antigen for use as immunogens. An antigenic peptide fragment comprises at least 6 amino acid residues of the amino acid sequence of the full length protein, such as an amino acid sequence shown in SEQ ID NO: 4 or 5, and encompasses an epitope thereof such that an antibody raised against the peptide forms a specific immune complex with the full length protein or with any fragment that contains the epitope. Preferably, the antigenic peptide comprises at least 10 amino acid residues, or at least 15 amino acid residues, or at least 20 amino acid residues, or at least 30 amino acid residues. Preferred epitopes encompassed by the antigenic peptide are regions of the protein that are located on its surface; commonly these are hydrophilic regions.

In certain embodiments of the invention, at least one epitope encompassed by the antigenic peptide is a surface region of the protein, *e.g.*, a hydrophilic region. A hydrophobicity analysis of the human related protein sequence will indicate which regions of a related protein are particularly hydrophilic and, therefore, are likely to encode surface residues useful for targeting antibody production. As a means for targeting antibody production, hydropathy plots showing regions of hydrophilicity and hydrophobicity may be generated by any method well known in the art, including, for example, the Kyte Doolittle or the Hopp Woods methods, either with or without Fourier transformation. See, *e.g.*, Hopp and Woods, 1981, Proc. Nat. Acad. Sci. USA 78: 3824-3828; Kyte and Doolittle 1982, J. Mol. Biol. 157: 105-142, each of which is incorporated herein by reference in its entirety. Antibodies that are specific for one or more domains within an antigenic protein, or derivatives, fragments, analogs or homologs thereof, are also provided herein.

A protein of the invention, or a derivative, fragment, analog, homolog or ortholog thereof, may be utilized as an immunogen in the generation of antibodies that immunospecifically bind these protein components.

The term "specific for" indicates that the variable regions of the antibodies of the invention recognize and bind polypeptides of the invention exclusively (*i.e.*, able to distinguish the polypeptide of the invention from other similar polypeptides despite sequence identity, homology, or similarity found in the family of polypeptides), but may also interact with other proteins (for example, *S. aureus* protein A or other antibodies in ELISA techniques) through interactions with sequences outside the variable region of the antibodies, and in particular, in the constant region of the molecule. Screening assays to determine binding specificity of an antibody of the invention are well known and routinely practiced in the art. For a comprehensive discussion of such assays, see Harlow et al. (Eds), Antibodies A Laboratory Manual; Cold Spring Harbor Laboratory; Cold Spring Harbor, NY (1988), Chapter 6. Antibodies that recognize and bind fragments of the polypeptides of the invention are also contemplated, provided that the antibodies are first and foremost specific for, as defined above, full-length polypeptides of the invention. As with antibodies that are specific for full length polypeptides of the invention, antibodies of the invention that recognize fragments are those which can distinguish polypeptides from the same family of polypeptides despite inherent sequence identity, homology, or similarity found in the family of proteins.

Antibodies of the invention are useful for, for example, therapeutic purposes (by modulating activity of a polypeptide of the invention), diagnostic purposes to detect or quantitate a polypeptide of the invention, as well as purification of a polypeptide of the invention. Kits comprising an antibody of the invention for any of the purposes described herein are also comprehended. In general, a kit of the invention also includes a control antigen for which the antibody is immunospecific. The invention further provides a hybridoma that produces an antibody according to the invention. Antibodies of the invention are useful for detection and/or purification of the polypeptides of the invention.

Monoclonal antibodies binding to the protein of the invention may be useful diagnostic agents for the immunodetection of the protein. Neutralizing monoclonal antibodies binding to the protein may also be useful therapeutics for both conditions

associated with the protein and also in the treatment of some forms of cancer where abnormal expression of the protein is involved. In the case of cancerous cells or leukemic cells, neutralizing monoclonal antibodies against the protein may be useful in detecting and preventing the metastatic spread of the cancerous cells, which may be mediated by the protein.

The labeled antibodies of the present invention can be used for *in vitro*, *in vivo*, and *in situ* assays to identify cells or tissues in which a fragment of the polypeptide of interest is expressed. The antibodies may also be used directly in therapies or other diagnostics. The present invention further provides the above-described antibodies immobilized on a solid support. Examples of such solid supports include plastics such as polycarbonate, complex carbohydrates such as agarose and Sepharose®, acrylic resins and such as polyacrylamide and latex beads. Techniques for coupling antibodies to such solid supports are well known in the art (Weir, D.M. et al., "Handbook of Experimental Immunology" 4th Ed., Blackwell Scientific Publications, Oxford, England, Chapter 10 (1986); Jacoby, W.D. et al., Meth. Enzym. 34 Academic Press, N.Y. (1974)). The immobilized antibodies of the present invention can be used for *in vitro*, *in vivo*, and *in situ* assays as well as for immuno-affinity purification of the proteins of the present invention.

Various procedures known within the art may be used for the production of polyclonal or monoclonal antibodies directed against a protein of the invention, or against derivatives, fragments, analogs homologs or orthologs thereof (see, for example, Antibodies: A Laboratory Manual, Harlow E, and Lane D, 1988, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, incorporated herein by reference). Some of these antibodies are discussed below.

4.7.1 POLYCLONAL ANTIBODIES

For the production of polyclonal antibodies, various suitable host animals (e.g., rabbit, goat, mouse or other mammal) may be immunized by one or more injections with the native protein, a synthetic variant thereof, or a derivative of the foregoing. An appropriate immunogenic preparation can contain, for example, the naturally occurring immunogenic protein, a chemically synthesized polypeptide representing the

immunogenic protein, or a recombinantly expressed immunogenic protein. Furthermore, the protein may be conjugated to a second protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. The preparation can further include an adjuvant. Various adjuvants used to increase the immunological response include, but are not limited to, Freund's (complete and incomplete), mineral gels (e.g., aluminum hydroxide), surface-active substances (e.g., lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, etc.), adjuvants usable in humans such as Bacille Calmette-Guerin and Corynebacterium parvum, or similar immunostimulatory agents. Additional examples of adjuvants that can be employed include MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate).

The polyclonal antibody molecules directed against the immunogenic protein can be isolated from the mammal (e.g., from the blood) and further purified by well known techniques, such as affinity chromatography using protein A or protein G, which provide primarily the IgG fraction of immune serum. Subsequently, or alternatively, the specific antigen which is the target of the immunoglobulin sought, or an epitope thereof, may be immobilized on a column to purify the immune specific antibody by immunoaffinity chromatography. Purification of immunoglobulins is discussed, for example, by D. Wilkinson (The Scientist, published by The Scientist, Inc., Philadelphia PA, Vol. 14, No. 8 (April 17, 2000), pp. 25-28).

4.7.2 MONOCLONAL ANTIBODIES

The term "monoclonal antibody" (MAb) or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one molecular species of antibody molecule consisting of a unique light chain gene product and a unique heavy chain gene product. In particular, the complementarity determining regions (CDRs) of the monoclonal antibody are identical in all the molecules of the population. MAbs thus contain an antigen-binding site capable of immunoreacting with a particular epitope of the antigen characterized by a unique binding affinity for it.

Monoclonal antibodies can be prepared using hybridoma methods, such as those described by Kohler and Milstein, Nature, 256, 495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes can be immunized in vitro.

The immunizing agent will typically include the protein antigen, a fragment thereof or a fusion protein thereof. Generally, either peripheral blood lymphocytes are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies: Principles and Practice, Academic Press, (1986) pp. 59-103). Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells can be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Manassas, Virginia. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York, (1987) pp. 51-63).

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollard, Anal. Biochem., 107, 220 (1980). Preferably, antibodies having a high degree of specificity and a high binding affinity for the target antigen are isolated.

After the desired hybridoma cells are identified, the clones can be subcloned by limiting dilution procedures and grown by standard methods. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells can be grown in vivo as ascites in a mammal.

The monoclonal antibodies secreted by the subclones can be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies can also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA can be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also can be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences (U.S. Patent No. 4,816,567; Morrison, Nature 368, 812-13 (1994)) or by covalently joining to the immunoglobulin coding sequence all

or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

4.7.3 HUMANIZED ANTIBODIES

The antibodies directed against the protein antigens of the invention can further comprise humanized antibodies or human antibodies. These antibodies are suitable for administration to humans without engendering an immune response by the human against the administered immunoglobulin. Humanized forms of antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies) that are principally comprised of the sequence of a human immunoglobulin, and contain minimal sequence derived from a non-human immunoglobulin. Humanization can be performed following the method of Winter and co-workers (Jones et al., Nature, 321, 522-525 (1986); Riechmann et al., Nature, 332, 323-327 (1988); Verhoeyen et al., Science, 239, 1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. (See also U.S. Patent No. 5,225,539). In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies can also comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin (Jones et al., 1986; Riechmann et al., 1988; and Presta, Curr. Op. Struct. Biol., 2, 593-596 (1992)).

4.7.4 HUMAN ANTIBODIES

Fully human antibodies relate to antibody molecules in which essentially the entire sequences of both the light chain and the heavy chain, including the CDRs, arise from human genes. Such antibodies are termed "human antibodies", or "fully human antibodies" herein. Human monoclonal antibodies can be prepared by the trioma technique; the human B-cell hybridoma technique (see Kozbor, et al., 1983 Immunol Today 4: 72) and the EBV hybridoma technique to produce human monoclonal antibodies (see Cole, et al., 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies may be utilized in the practice of the present invention and may be produced by using human hybridomas (see Cote, et al., 1983. Proc Natl Acad Sci USA 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus in vitro (see Cole, et al., 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96).

In addition, human antibodies can also be produced using additional techniques, including phage display libraries (Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991)). Similarly, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in Marks et al. (Bio/Technology 10, 779-783 (1992)); Lonberg et al. (Nature 368 856-859 (1994)); Morrison (Nature 368, 812-13 (1994)); Fishwild et al,(Nature Biotechnology 14, 845-51 (1996)); Neuberger (Nature Biotechnology 14, 826 (1996)); and Lonberg and Huszar (Intern. Rev. Immunol. 13 65-93 (1995)).

Human antibodies may additionally be produced using transgenic nonhuman animals which are modified so as to produce fully human antibodies rather than the animal's endogenous antibodies in response to challenge by an antigen. (See PCT publication WO94/02602). The endogenous genes encoding the heavy and light immunoglobulin chains in the nonhuman host have been incapacitated, and active loci encoding human heavy and light chain immunoglobulins are inserted into the host's

genome. The human genes are incorporated, for example, using yeast artificial chromosomes containing the requisite human DNA segments. An animal which provides all the desired modifications is then obtained as progeny by crossbreeding intermediate transgenic animals containing fewer than the full complement of the modifications. The preferred embodiment of such a nonhuman animal is a mouse, and is termed the Xenomouse™ as disclosed in PCT publications WO 96/33735 and WO 96/34096. This animal produces B cells which secrete fully human immunoglobulins. The antibodies can be obtained directly from the animal after immunization with an immunogen of interest, as, for example, a preparation of a polyclonal antibody, or alternatively from immortalized B cells derived from the animal, such as hybridomas producing monoclonal antibodies. Additionally, the genes encoding the immunoglobulins with human variable regions can be recovered and expressed to obtain the antibodies directly, or can be further modified to obtain analogs of antibodies such as, for example, single chain Fv molecules.

An example of a method of producing a nonhuman host, exemplified as a mouse, lacking expression of an endogenous immunoglobulin heavy chain is disclosed in U.S. Patent No. 5,939,598. It can be obtained by a method including deleting the J segment genes from at least one endogenous heavy chain locus in an embryonic stem cell to prevent rearrangement of the locus and to prevent formation of a transcript of a rearranged immunoglobulin heavy chain locus, the deletion being effected by a targeting vector containing a gene encoding a selectable marker; and producing from the embryonic stem cell a transgenic mouse whose somatic and germ cells contain the gene encoding the selectable marker.

A method for producing an antibody of interest, such as a human antibody, is disclosed in U.S. Patent No. 5,916,771. It includes introducing an expression vector that contains a nucleotide sequence encoding a heavy chain into one mammalian host cell in culture, introducing an expression vector containing a nucleotide sequence encoding a light chain into another mammalian host cell, and fusing the two cells to form a hybrid cell. The hybrid cell expresses an antibody containing the heavy chain and the light chain.

In a further improvement on this procedure, a method for identifying a clinically relevant epitope on an immunogen, and a correlative method for selecting an antibody

that binds immunospecifically to the relevant epitope with high affinity, are disclosed in PCT publication WO 99/53049.

4.7.5 FAB FRAGMENTS AND SINGLE CHAIN ANTIBODIES

According to the invention, techniques can be adapted for the production of single-chain antibodies specific to an antigenic protein of the invention (see e.g., U.S. Patent No. 4,946,778). In addition, methods can be adapted for the construction of F_{ab} expression libraries (see e.g., Huse, et al., 1989 Science 246: 1275-1281) to allow rapid and effective identification of monoclonal F_{ab} fragments with the desired specificity for a protein or derivatives, fragments, analogs or homologs thereof. Antibody fragments that contain the idiotypes to a protein antigen may be produced by techniques known in the art including, but not limited to: (i) an $F_{(ab')2}$ fragment produced by pepsin digestion of an antibody molecule; (ii) an F_{ab} fragment generated by reducing the disulfide bridges of an $F_{(ab')2}$ fragment; (iii) an F_{ab} fragment generated by the treatment of the antibody molecule with papain and a reducing agent and (iv) F_v fragments.

4.7.6 BISPECIFIC ANTIBODIES

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for an antigenic protein of the invention. The second binding target is any other antigen, and advantageously is a cell-surface protein or receptor or receptor subunit.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, Nature, 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed

in WO 93/08829, published 13 May 1993, and in Traunecker *et al.*, 1991 *EMBO J.*, 10:3655-3659.

Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. For further details of generating bispecific antibodies see, for example, Suresh et al., Methods in Enzymology, 121:210 (1986).

According to another approach described in WO 96/27011, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the CH3 region of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies can be prepared as full-length antibodies or antibody fragments (e.g. $F(ab')_2$ bispecific antibodies). Techniques for generating bispecific antibodies from antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan et al., Science 229:81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate $F(ab')_2$ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to

thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Additionally, Fab' fragments can be directly recovered from E. coli and chemically coupled to form bispecific antibodies. Shalaby et al., J. Exp. Med. 175:217-225 (1992) describe the production of a fully humanized bispecific antibody $F(ab')_2$ molecule. Each Fab' fragment was separately secreted from E. coli and subjected to directed chemical coupling in vitro to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny et al., J. Immunol. 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger et al., Proc. Natl. Acad. Sci. USA 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See, Gruber et al., J. Immunol. 152:5368 (1994).

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt et al., J. Immunol. 147:60 (1991).

Exemplary bispecific antibodies can bind to two different epitopes, at least one of which originates in the protein antigen of the invention. Alternatively, an anti-antigenic arm of an immunoglobulin molecule can be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (e.g. CD2, CD3, CD28, or B7), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the cell expressing the particular antigen. Bispecific antibodies can also be used to direct cytotoxic agents to cells which express a particular antigen. These antibodies possess an antigen-binding arm and an arm which binds a cytotoxic agent or a radionuclide chelator, such as EOTUBE, DPTA, DOTA, or TETA. Another bispecific antibody of interest binds the protein antigen described herein and further binds tissue factor (TF).

4.7.7 HETEROCONJUGATE ANTIBODIES

Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360; WO 92/200373; EP 03089). It is contemplated that the antibodies can be prepared in vitro using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins can be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

4.7.8 EFFECTOR FUNCTION ENGINEERING

It can be desirable to modify the antibody of the invention with respect to effector function, so as to enhance, e.g., the effectiveness of the antibody in treating cancer. For example, cysteine residue(s) can be introduced into the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated can have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC).

See Caron et al., J. Exp Med., 176: 1191-1195 (1992) and Shopes, J. Immunol., 148: 2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity can also be prepared using heterobifunctional cross-linkers as described in Wolff et al. Cancer Research, 53: 2560-2565 (1993). Alternatively, an antibody can be engineered that has dual Fc regions and can thereby have enhanced complement lysis and ADCC capabilities. See Stevenson et al., Anti-Cancer Drug Design, 3: 219-230 (1989).

4.7.9 IMMUNOCONJUGATES

The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (e.g., an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (i.e., a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include ^{212}Bi, ^{131}I, ^{131}In, ^{90}Y, and ^{186}Re.

Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science, 238: 1098 (1987).

Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026.

In another embodiment, the antibody can be conjugated to a "receptor" (such streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g., avidin) that is in turn conjugated to a cytotoxic agent.

4.8 USES AND BIOLOGICAL ACTIVITY OF HUMAN JPL POLYPEPTIDE

The polynucleotides and proteins of the present invention are expected to exhibit one or more of the uses or biological activities (including those associated with assays cited herein) identified herein. Uses or activities described for proteins of the present invention may be provided by administration or use of such proteins or of polynucleotides encoding such proteins (such as, for example, in gene therapies or vectors suitable for introduction of DNA). The mechanism underlying the particular condition or pathology will dictate whether the polypeptides of the invention, the polynucleotides of the invention or modulators (activators or inhibitors) thereof would be beneficial to the subject in need of treatment. Thus, "therapeutic compositions of the invention" include compositions comprising isolated polynucleotides (including recombinant DNA molecules, cloned genes and degenerate variants thereof) or polypeptides of the invention (including full length protein, mature protein and truncations or domains thereof), or compounds and other substances that modulate the overall activity of the target gene products, either at the level of target gene/protein expression or target protein activity. Such modulators include polypeptides, analogs, (variants), including fragments and fusion proteins, antibodies and other binding proteins; chemical compounds that directly or indirectly activate or inhibit the polypeptides of the invention (identified, e.g., via drug screening assays as described herein); antisense polynucleotides and polynucleotides suitable for triple helix formation; and in particular

antibodies or other binding partners that specifically recognize one or more epitopes of the polypeptides of the invention.

The polypeptides of the present invention may likewise be involved in cellular activation or in one of the other physiological pathways described herein.

4.8.1 RESEARCH USES AND UTILITIES

The polynucleotides provided by the present invention can be used by the research community for various purposes. The polynucleotides can be used to express recombinant protein for analysis, characterization or therapeutic use; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in disease states); as molecular weight markers on gels; as chromosome markers or tags (when labeled) to identify chromosomes or to map related gene positions; to compare with endogenous DNA sequences in patients to identify potential genetic disorders; as probes to hybridize and thus discover novel, related DNA sequences; as a source of information to derive PCR primers for genetic fingerprinting; as a probe to "subtract-out" known sequences in the process of discovering other novel polynucleotides; for selecting and making oligomers for attachment to a "gene chip" or other support, including for examination of expression patterns; to raise anti-protein antibodies using DNA immunization techniques; and as an antigen to raise anti-DNA antibodies or elicit another immune response. Where the polynucleotide encodes a protein which binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the polynucleotide can also be used in interaction trap assays (such as, for example, that described in Gyuris et al., Cell 75:791-803 (1993)) to identify polynucleotides encoding the other protein with which binding occurs or to identify inhibitors of the binding interaction.

The polypeptides provided by the present invention can similarly be used in assays to determine biological activity, including in a panel of multiple proteins for high-throughput screening; to raise antibodies or to elicit another immune response; as a reagent (including the labeled reagent) in assays designed to quantitatively determine levels of the protein (or its receptor) in biological fluids; as markers for tissues in which

the corresponding polypeptide is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in a disease state); and, of course, to isolate correlative receptors or ligands. Proteins involved in these binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction.

The polypeptides of the invention are also useful for making antibody substances that are specifically immunoreactive with JPL proteins. Antibodies and portions thereof (e.g., Fab fragments) which bind to the polypeptides of the invention can be used to identify the presence of such polypeptides in a sample. Such determinations are carried out using any suitable immunoassay format, and any polypeptide of the invention that is specifically bound by the antibody can be employed as a positive control.

Any or all of these research utilities are capable of being developed into reagent grade or kit format for commercialization as research products.

Methods for performing the uses listed above are well known to those skilled in the art. References disclosing such methods include without limitation "Molecular Cloning: A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory Press, Sambrook, J., E. F. Fritsch and T. Maniatis eds., 1989, and "Methods in Enzymology: Guide to Molecular Cloning Techniques", Academic Press, Berger, S. L. and A. R. Kimmel eds., 1987.

4.8.2 CYTOKINE AND CELL PROLIFERATION/DIFFERENTIATION ACTIVITY

A polypeptide of the present invention may exhibit activity relating to cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. A polynucleotide of the invention can encode a polypeptide exhibiting such attributes. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor-dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of therapeutic compositions of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D,

DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+(preB M+), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e, CMK, HUVEC, and Caco. Therapeutic compositions of the invention can be used in the following:

Assays for T-cell or thymocyte proliferation include without limitation those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, *In Vitro* assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Bertagnolli et al., J. Immunol. 145:1706-1712, 1990; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Bertagnolli, et al., I. Immunol. 149:3778-3783, 1992; Bowman et al., I. Immunol. 152:1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described in: Polyclonal T cell stimulation, Kruisbeek, A. M. and Shevach, E. M. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 3.12.1-3.12.14, John Wiley and Sons, Toronto. 1994; and Measurement of mouse and human interferon-γ, Schreiber, R. D. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto. 1994.

Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described in: Measurement of Human and Murine Interleukin 2 and Interleukin 4, Bottomly, K., Davis, L. S. and Lipsky, P. E. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto. 1991; deVries et al., J. Exp. Med. 173:1205-1211, 1991; Moreau et al., Nature 336:690-692, 1988; Greenberger et al., Proc. Natl. Acad. Sci. U.S.A. 80:2931-2938, 1983; Measurement of mouse and human interleukin 6--Nordan, R. In Current Protocols in Immunology. J. E. Coligan eds. Vol 1 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto. 1991; Smith et al., Proc. Natl. Aced. Sci. U.S.A. 83:1857-1861, 1986; Measurement of human Interleukin 11--Bennett, F., Giannotti, J., Clark, S. C. and Turner, K. J. In Current Protocols in Immunology. J. E. Coligan eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto. 1991; Measurement of mouse and human Interleukin 9-

Ciarletta, A., Giannotti, J., Clark, S. C. and Turner, K. J. In Current Protocols in Immunology. J. E. Coligan eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto. 1991.

Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, *In Vitro* assays for Mouse Lymphocyte Function; Chapter 6, Cytokines and their cellular receptors; Chapter 7, Immunologic studies in Humans); Weinberger et al., Proc. Natl. Acad. Sci. USA 77:6091-6095, 1980; Weinberger et al., Eur. J. Immun. 11:405-411, 1981; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988.

4.8.3 STEM CELL GROWTH FACTOR ACTIVITY

A polypeptide of the present invention may exhibit stem cell growth factor activity and be involved in the proliferation, differentiation and survival of pluripotent and totipotent stem cells including primordial germ cells, embryonic stem cells, hematopoietic stem cells and/or germ line stem cells. Administration of the polypeptide of the invention to stem cells *in vivo* or *ex vivo* may maintain and expand cell populations in a totipotential or pluripotential state which would be useful for re-engineering damaged or diseased tissues, transplantation, manufacture of bio-pharmaceuticals and the development of bio-sensors. The ability to produce large quantities of human cells has important working applications for the production of human proteins which currently must be obtained from non-human sources or donors, implantation of cells to treat diseases such as Parkinson's, Alzheimer's and other neurodegenerative diseases; tissues for grafting such as bone marrow, skin, cartilage, tendons, bone, muscle (including cardiac muscle), blood vessels, cornea, neural cells, gastrointestinal cells and others; and organs for transplantation such as kidney, liver, pancreas (including islet cells), heart and lung.

It is contemplated that multiple different exogenous growth factors and/or cytokines may be administered in combination with the polypeptide of the invention to

achieve the desired effect, including any of the growth factors listed herein, other stem cell maintenance factors, and specifically including stem cell factor (SCF), leukemia inhibitory factor (LIF), Flt-3 ligand (Flt-3L), any of the interleukins, recombinant soluble IL-6 receptor fused to IL-6, macrophage inflammatory protein 1-alpha (MIP-1-alpha), G-CSF, GM-CSF, thrombopoietin (TPO), platelet factor 4 (PF-4), platelet-derived growth factor (PDGF), neural growth factors and basic fibroblast growth factor (bFGF).

Since totipotent stem cells can give rise to virtually any mature cell type, expansion of these cells in culture will facilitate the production of large quantities of mature cells. Techniques for culturing stem cells are known in the art and administration of polypeptides of the invention, optionally with other growth factors and/or cytokines, is expected to enhance the survival and proliferation of the stem cell populations. This can be accomplished by direct administration of the polypeptide of the invention to the culture medium. Alternatively, stroma cells transfected with a polynucleotide that encodes for the polypeptide of the invention can be used as a feeder layer for the stem cell populations in culture or in vivo. Stromal support cells for feeder layers may include embryonic bone marrow fibroblasts, bone marrow stromal cells, fetal liver cells, or cultured embryonic fibroblasts (see U.S. Patent No. 5,690,926).

Stem cells themselves can be transfected with a polynucleotide of the invention to induce autocrine expression of the polypeptide of the invention. This will allow for generation of undifferentiated totipotential/pluripotential stem cell lines that are useful as is or that can then be differentiated into the desired mature cell types. These stable cell lines can also serve as a source of undifferentiated totipotential/pluripotential mRNA to create cDNA libraries and templates for polymerase chain reaction experiments. These studies would allow for the isolation and identification of differentially expressed genes in stem cell populations that regulate stem cell proliferation and/or maintenance.

Expansion and maintenance of totipotent stem cell populations will be useful in the treatment of many pathological conditions. For example, polypeptides of the present invention may be used to manipulate stem cells in culture to give rise to neuroepithelial cells that can be used to augment or replace cells damaged by illness, autoimmune disease, accidental damage or genetic disorders. The polypeptide of the invention may be useful for inducing the proliferation of neural cells and for the regeneration of nerve and

brain tissue, i.e. for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders which involve degeneration, death or trauma to neural cells or nerve tissue. Furthermore, these cells can be cultured *in vitro* to form other differentiated cells, such as skin tissue that can be used for transplantation. In addition, the expanded stem cell populations can also be genetically altered for gene therapy purposes and to decrease host rejection of replacement tissues after grafting or implantation.

Expression of the polypeptide of the invention and its effect on stem cells can also be manipulated to achieve controlled differentiation of the stem cells into more differentiated cell types. A broadly applicable method of obtaining pure populations of a specific differentiated cell type from undifferentiated stem cell populations involves the use of a cell-type specific promoter driving a selectable marker. The selectable marker allows only cells of the desired type to survive. For example, stem cells can be induced to differentiate into cardiomyocytes (Wobus et al., Differentiation, 48: 173-182, (1991); Klug et al., J. Clin. Invest., 98(1): 216-224, (1998)) or skeletal muscle cells (Browder, L. W. In: *Principles of Tissue Engineering eds.* Lanza et al., Academic Press (1997)). Alternatively, directed differentiation of stem cells can be accomplished by culturing the stem cells in the presence of a differentiation factor such as retinoic acid and an antagonist of the polypeptide of the invention which would inhibit the effects of endogenous stem cell factor activity and allow differentiation to proceed.

In vitro cultures of stem cells can be used to determine if the polypeptide of the invention exhibits stem cell growth factor activity. Stem cells are isolated from any one of various cell sources (including hematopoietic stem cells and embryonic stem cells) and cultured on a feeder layer, as described by Thompson et al. Proc. Natl. Acad. Sci, U.S.A., 92: 7844-7848 (1995), in the presence of the polypeptide of the invention alone or in combination with other growth factors or cytokines. The ability of the polypeptide of the invention to induce stem cells proliferation is determined by colony formation on semi-solid support e.g. as described by Bernstein et al., Blood, 77: 2316-2321 (1991).

4.8.4 IMMUNE FUNCTION STIMULATING OR SUPPRESSING ACTIVITY

A polypeptide of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A polynucleotide of the invention can encode a polypeptide exhibiting such activities. A protein may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (e.g., HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases causes by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpes viruses, mycobacteria, Leishmania spp., malaria spp. and various fungal infections such as candidiasis. Of course, in this regard, proteins of the present invention may also be useful where a boost to the immune system generally may be desirable, i.e., in the treatment of cancer.

Autoimmune disorders which may be treated using a protein of the present invention include, for example, connective tissue disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitis, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein (or antagonists thereof, including antibodies) of the present invention may also to be useful in the treatment of allergic reactions and conditions (*e.g.*, anaphylaxis, serum sickness, drug reactions, food allergies, insect venom allergies, mastocytosis, allergic rhinitis, hypersensitivity pneumonitis, urticaria, angioedema, eczema, atopic dermatitis, allergic contact dermatitis, erythema multiforme, Stevens-Johnson syndrome, allergic conjunctivitis, atopic keratoconjunctivitis, venereal keratoconjunctivitis, giant papillary conjunctivitis and contact allergies), such as asthma (particularly allergic asthma) or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, organ transplantation), may also be treatable using a protein (or antagonists thereof) of the present invention. The therapeutic effects of the polypeptides or antagonists thereof on allergic reactions can be evaluated by in vivo

animals models such as the cumulative contact enhancement test (Lastbom et al., Toxicology 125: 59-66, 1998), skin prick test (Hoffmann et al., Allergy 54: 446-54, 1999), guinea pig skin sensitization test (Vohr et al., Arch. Toxocol. 73: 501-9), and murine local lymph node assay (Kimber et al., J. Toxicol. Environ. Health 53: 563-79).

Using the proteins of the invention it may also be possible to modulate immune responses, in a number of ways. Down regulation may be in the form of inhibiting or blocking an immune response already in progress or may involve preventing the induction of an immune response. The functions of activated T cells may be inhibited by suppressing T cell responses or by inducing specific tolerance in T cells, or both. Immunosuppression of T cell responses is generally an active, non-antigen-specific, process which requires continuous exposure of the T cells to the suppressive agent. Tolerance, which involves inducing non-responsiveness or anergy in T cells, is distinguishable from immunosuppression in that it is generally antigen-specific and persists after exposure to the tolerizing agent has ceased. Operationally, tolerance can be demonstrated by the lack of a T cell response upon reexposure to specific antigen in the absence of the tolerizing agent.

Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions (such as, for example, B7)), e.g., preventing high level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The administration of a therapeutic composition of the invention may prevent cytokine synthesis by immune cells, such as T cells, and thus acts as an immunosuppressant. Moreover, a lack of costimulation may also be sufficient to anergize the T cells, thereby inducing tolerance in a subject. Induction of long-term tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or tolerance in a subject, it may also be necessary to block the function of a combination of B lymphocyte antigens.

The efficacy of particular therapeutic compositions in preventing organ transplant rejection or GVHD can be assessed using animal models that are predictive of efficacy in humans. Examples of appropriate systems which can be used include allogeneic cardiac grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive effects of CTLA4Ig fusion proteins in vivo as described in Lenschow et al., Science 257:789-792 (1992) and Turka et al., Proc. Natl. Acad. Sci USA, 89:11102-11105 (1992). In addition, murine models of GVHD (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 846-847) can be used to determine the effect of therapeutic compositions of the invention on the development of that disease.

Blocking antigen function may also be therapeutically useful for treating autoimmune diseases. Many autoimmune disorders are the result of inappropriate activation of T cells that are reactive against self tissue and which promote the production of cytokines and autoantibodies involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate disease symptoms. Administration of reagents which block stimulation of T cells can be used to inhibit T cell activation and prevent production of autoantibodies or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of autoreactive T cells which could lead to long-term relief from the disease. The efficacy of blocking reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythematosus in MRL/lpr/lpr mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and murine experimental myasthenia gravis (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 840-856).

Upregulation of an antigen function (e.g., a B lymphocyte antigen function), as a means of up regulating immune responses, may also be useful in therapy. Upregulation of immune responses may be in the form of enhancing an existing immune response or eliciting an initial immune response. For example, enhancing an immune response may

be useful in cases of viral infection, including systemic viral diseases such as influenza, the common cold, and encephalitis.

Alternatively, anti-viral immune responses may be enhanced in an infected patient by removing T cells from the patient, costimulating the T cells in vitro with viral antigen-pulsed APCs either expressing a peptide of the present invention or together with a stimulatory form of a soluble peptide of the present invention and reintroducing the in vitro activated T cells into the patient. Another method of enhancing anti-viral immune responses would be to isolate infected cells from a patient, transfect them with a nucleic acid encoding a protein of the present invention as described herein such that the cells express all or a portion of the protein on their surface, and reintroduce the transfected cells into the patient. The infected cells would now be capable of delivering a costimulatory signal to, and thereby activate, T cells in vivo.

A polypeptide of the present invention may provide the necessary stimulation signal to T cells to induce a T cell mediated immune response against the transfected tumor cells. In addition, tumor cells which lack MHC class I or MHC class II molecules, or which fail to reexpress sufficient mounts of MHC class I or MHC class II molecules, can be transfected with nucleic acid encoding all or a portion of (e.g., a cytoplasmic-domain truncated portion) of an MHC class I alpha chain protein and β_2 microglobulin protein or an MHC class II alpha chain protein and an MHC class II beta chain protein to thereby express MHC class I or MHC class II proteins on the cell surface. Expression of the appropriate class I or class II MHC in conjunction with a peptide having the activity of a B lymphocyte antigen (e.g., B7-1, B7-2, B7-3) induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II associated protein, such as the invariant chain, can also be cotransfected with a DNA encoding a peptide having the activity of a B lymphocyte antigen to promote presentation of tumor associated antigens and induce tumor specific immunity. Thus, the induction of a T cell mediated immune response in a human subject may be sufficient to overcome tumor-specific tolerance in the subject.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., I. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Bowman et al., J. Virology 61:1992-1998; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Brown et al., J. Immunol. 153:3079-3092, 1994.

Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J. Immunol. 144:3028-3033, 1990; and Assays for B cell function: In vitro antibody production, Mond, J. J. and Brunswick, M. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto. 1994.

Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins that generate predominantly Th1 and CTL responses) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., J. Immunol. 149:3778-3783, 1992.

Dendritic cell-dependent assays (which will identify, among others, proteins expressed by dendritic cells that activate naive T-cells) include, without limitation, those described in: Guery et al., J. Immunol. 134:536-544, 1995; Inaba et al., Journal of Experimental Medicine 173:549-559, 1991; Macatonia et al., Journal of Immunology 154:5071-5079, 1995; Porgador et al., Journal of Experimental Medicine 182:255-260, 1995; Nair et al., Journal of Virology 67:4062-4069, 1993; Huang et al., Science

264:961-965, 1994; Macatonia et al., Journal of Experimental Medicine 169:1255-1264, 1989; Bhardwaj et al., Journal of Clinical Investigation 94:797-807, 1994; and Inaba et al., Journal of Experimental Medicine 172:631-640, 1990.

Assays for lymphocyte survival/apoptosis (which will identify, among others, proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz et al., Cytometry 13:795-808, 1992; Gorczyca et al., Leukemia 7:659-670, 1993; Gorczyca et al., Cancer Research 53:1945-1951, 1993; Itoh et al., Cell 66:233-243, 1991; Zacharchuk, Journal of Immunology 145:4037-4045, 1990; Zamai et al., Cytometry 14:891-897, 1993; Gorczyca et al., International Journal of Oncology 1:639-648, 1992.

Assays for proteins that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica et al., Blood 84:111-117, 1994; Fine et al., Cellular Immunology 155:111-122, 1994; Galy et al., Blood 85:2770-2778, 1995; Toki et al., Proc. Nat. Acad Sci. USA 88:7548-7551, 1991.

4.8.5 CHEMOTACTIC/CHEMOKINETIC ACTIVITY

A polypeptide of the present invention may be involved in chemotactic or chemokinetic activity for mammalian cells, including, for example, monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells. A polynucleotide of the invention can encode a polypeptide exhibiting such attributes. Chemotactic and chemokinetic receptor activation can be used to mobilize or attract a desired cell population to a desired site of action. Chemotactic or chemokinetic compositions (e.g. proteins, antibodies, binding partners, or modulators of the invention) provide particular advantages in treatment of wounds and other trauma to tissues, as well as in treatment of localized infections. For example, attraction of lymphocytes, monocytes or neutrophils to tumors or sites of infection may result in improved immune responses against the tumor or infecting agent.

A protein or peptide has chemotactic activity for a particular cell population if it can stimulate, directly or indirectly, the directed orientation or movement of such cell population. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a population

of cells can be readily determined by employing such protein or peptide in any known assay for cell chemotaxis.

Therapeutic compositions of the invention can be used in the following:

Assays for chemotactic activity (which will identify proteins that induce or prevent chemotaxis) consist of assays that measure the ability of a protein to induce the migration of cells across a membrane as well as the ability of a protein to induce the adhesion of one cell population to another cell population. Suitable assays for movement and adhesion include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Marguiles, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 6.12, Measurement of alpha and beta Chemokines 6.12.1-6.12.28; Taub et al. J. Clin. Invest. 95:1370-1376, 1995; Lind et al. APMIS 103:140-146, 1995; Muller et al Eur. J. Immunol. 25:1744-1748; Gruber et al. J. of Immunol. 152:5860-5867, 1994; Johnston et al. J. of Immunol. 153:1762-1768, 1994.

4.8.6 CANCER DIAGNOSIS AND THERAPY

Polypeptides of the invention may be involved in cancer cell generation, proliferation or metastasis. Detection of the presence or amount of polynucleotides or polypeptides of the invention may be useful for the diagnosis and/or prognosis of one or more types of cancer. For example, the presence or increased expression of a polynucleotide/polypeptide of the invention may indicate a hereditary risk of cancer, a precancerous condition, or an ongoing malignancy. Conversely, a defect in the gene or absence of the polypeptide may be associated with a cancer condition. Identification of single nucleotide polymorphisms associated with cancer or a predisposition to cancer may also be useful for diagnosis or prognosis.

Cancer treatments promote tumor regression by inhibiting tumor cell proliferation, inhibiting angiogenesis (growth of new blood vessels that is necessary to support tumor growth) and/or prohibiting metastasis by reducing tumor cell motility or invasiveness. Therapeutic compositions of the invention may be effective in adult and pediatric oncology including in solid phase tumors/malignancies, locally advanced tumors, human soft tissue sarcomas, metastatic cancer, including lymphatic metastases,

blood cell malignancies including multiple myeloma, acute and chronic leukemias, and lymphomas, head and neck cancers including mouth cancer, larynx cancer and thyroid cancer, lung cancers including small cell carcinoma and non-small cell cancers, breast cancers including small cell carcinoma and ductal carcinoma, gastrointestinal cancers including esophageal cancer, stomach cancer, colon cancer, colorectal cancer and polyps associated with colorectal neoplasia, pancreatic cancers, liver cancer, urologic cancers including bladder cancer and prostate cancer, malignancies of the female genital tract including ovarian carcinoma, uterine (including endometrial) cancers, and solid tumor in the ovarian follicle, kidney cancers including renal cell carcinoma, brain cancers including intrinsic brain tumors, neuroblastoma, astrocytic brain tumors, gliomas, metastatic tumor cell invasion in the central nervous system, bone cancers including osteomas, skin cancers including malignant melanoma, tumor progression of human skin keratinocytes, squamous cell carcinoma, basal cell carcinoma, hemangiopericytoma and Karposi's sarcoma.

Polypeptides, polynucleotides, or modulators of polypeptides of the invention (including inhibitors and stimulators of the biological activity of the polypeptide of the invention) may be administered to treat cancer. Therapeutic compositions can be administered in therapeutically effective dosages alone or in combination with adjuvant cancer therapy such as surgery, chemotherapy, radiotherapy, thermotherapy, and laser therapy, and may provide a beneficial effect, e.g. reducing tumor size, slowing rate of tumor growth, inhibiting metastasis, or otherwise improving overall clinical condition, without necessarily eradicating the cancer.

The composition can also be administered in therapeutically effective amounts as a portion of an anti-cancer cocktail. An anti-cancer cocktail is a mixture of the polypeptide or modulator of the invention with one or more anti-cancer drugs in addition to a pharmaceutically acceptable carrier for delivery. The use of anti-cancer cocktails as a cancer treatment is routine. Anti-cancer drugs that are well known in the art and can be used as a treatment in combination with the polypeptide or modulator of the invention include: Actinomycin D, Aminoglutethimide, Asparaginase, Bleomycin, Busulfan, Carboplatin, Carmustine, Chlorambucil, Cisplatin (cis-DDP), Cyclophosphamide, Cytarabine HCl (Cytosine arabinoside), Dacarbazine, Dactinomycin,

Daunorubicin HCl, Doxorubicin HCl, Estramustine phosphate sodium, Etoposide (V16-213), Floxuridine, 5-Fluorouracil (5-Fu), Flutamide, Hydroxyurea (hydroxycarbamide), Ifosfamide, Interferon Alpha-2a, Interferon Alpha-2b, Leuprolide acetate (LHRH-releasing factor analog), Lomustine, Mechlorethamine HCl (nitrogen mustard), Melphalan, Mercaptopurine, Mesna, Methotrexate (MTX), Mitomycin, Mitoxantrone HCl, Octreotide, Plicamycin, Procarbazine HCl, Streptozocin, Tamoxifen citrate, Thioguanine, Thiotepa, Vinblastine sulfate, Vincristine sulfate, Amsacrine, Azacitidine, Hexamethylmelamine, Interleukin-2, Mitoguazone, Pentostatin, Semustine, Teniposide, and Vindesine sulfate.

In addition, therapeutic compositions of the invention may be used for prophylactic treatment of cancer. There are hereditary conditions and/or environmental situations (e.g. exposure to carcinogens) known in the art that predispose an individual to developing cancers. Under these circumstances, it may be beneficial to treat these individuals with therapeutically effective doses of the polypeptide of the invention to reduce the risk of developing cancers.

In vitro models can be used to determine the effective doses of the polypeptide of the invention as a potential cancer treatment. These *in vitro* models include proliferation assays of cultured tumor cells, growth of cultured tumor cells in soft agar (see Freshney, (1987) Culture of Animal Cells: A Manual of Basic Technique, Wily-Liss, New York, NY Ch 18 and Ch 21), tumor systems in nude mice as described in Giovanella et al., J. Natl. Can. Inst., 52: 921-30 (1974), mobility and invasive potential of tumor cells in Boyden Chamber assays as described in Pilkington et al., Anticancer Res., 17: 4107-9 (1997), and angiogenesis assays such as induction of vascularization of the chick chorioallantoic membrane or induction of vascular endothelial cell migration as described in Ribatta et al., Intl. J. Dev. Biol., 40: 1189-97 (1999) and Li et al., Clin. Exp. Metastasis, 17:423-9 (1999), respectively. Suitable tumor cells lines are available, e.g. from American Type Tissue Culture Collection catalogs.

4.8.7 TARGETING OF JPL POLYPEPTIDES

The amino acid sequence of an exemplary polypeptide and the nucleic acid sequence of the cDNA encoding the polypeptide are provided in SEQ ID NOS: 5 and 3, respectively. The data described herein in Example 4 show SEQ ID NO: 5 is expressed in melanoma tumors and in tissue adjacent to the melanoma tumors, while other healthy tissues fail to express JPL (SEQ ID NO: 5). Thus, targeting JPL polypeptides and fragments thereof will have a minimal effect on healthy tissue while destroying or inhibiting the growth of the melanoma cancer cells.

4.8.7.1 TARGETING USING JPL POLYPEPTIDE VACCINES

One embodiment the present invention provides a vaccine comprising a JPL polypeptide to stimulate the immune system against JPL polypeptides, thus targeting JPL polypeptide-expressing cells. Use of a tumor antigen in a vaccine for generating cellular and humoral immunity for the purpose of anti-cancer therapy is well known in the art. For example, one type of tumor-specific vaccine uses purified idiotype protein isolated from tumor cells, coupled to keyhole limpet hemocyanin (KLH) and mixed with adjuvant for injection into patients with low-grade follicular lymphoma (Hsu, *et al.*, *Blood* 89: 3129-3135 (1997)). U.S. Patent No. 6,312,718 describes methods for inducing immune responses against malignant B cells, in particular lymphoma, chronic lymphocytic leukemia, and multiple myeloma. The methods described therein utilize vaccines that include liposomes having (1) at least one B-cell malignancy-associated antigen, (2) IL-2 alone, or in combination with at least one other cytokine or chemokine, and (3) at least one lipid molecule. Methods of vaccinating against JPL polypeptides typically employ a JPL polypeptide, including fragments, analogs and variants.

As another example, dendritic cells, one type of antigen-presenting cell, can be used in a cellular vaccine in which the dendritic cells are isolated from the patient, co-cultured with tumor antigen and then reinfused as a cellular vaccine (Hsu, *et al.*, *Nat. Med.* 2:52-58 (1996)).

Combining this vaccine therapy with other types of therapeutic agents in treatments such as chemotherapy or radiotherapy is also contemplated.

4.8.7.2 TARGETING USING JPL NUCLEIC ACIDS

A. DIRECT DELIVERY OF NUCLEIC ACIDS

However, in some embodiments, a nucleic acid encoding JPL polypeptides, or encoding a fragment, analog or variant thereof, within a recombinant vector is utilized. Such methods are known in the art. For example, immune responses can be induced by injection of naked DNA. Plasmid DNA that expresses bicistronic mRNA encoding both the light and heavy chains of tumor idiotype proteins, such as those from B cell lymphoma, when injected into mice, are able to generate a protective, anti-tumor response (Singh, *et al.*, *Vaccine* 20:1400-1411 (2002)). JPL polypeptide viral vectors are particularly useful for delivering JPL polypeptide-encoding nucleic acids to cells. Examples of vectors include those derived from influenza, adenovirus, vaccinia, herpes symplex virus, fowlpox, vesicular stomatitis virus, canarypox, poliovirus, adeno-associated virus, and lentivirus and sindbus virus. Of course, non-viral vectors, such as liposomes or even naked DNA, are also useful for delivering JPL polypeptide-encoding nucleic acids to cells.

Combining this type of therapy with other types of therapeutic agents or treatments such as chemotherapy or radiation is also contemplated.

B. JPL POLYPEPTIDE NUCLEIC ACIDS EXPRESSED IN CELLS

In some embodiments, a vector comprising a nucleic acid encoding the JPL polypeptide (including a fragment, analog or variant) is introduced into a cell, such as a dendritic cell or a macrophage. When expressed in an antigen-presenting cell, JPL antigens are presented to T cells eliciting an immune response against JPL polypeptides. Such methods are also known in the art. Methods of introducing tumor antigens into antigen presenting cells and vectors useful therefore are described in U.S. Patent No. 6,300,090. The vector encoding JPL polypeptides may be introduced into the antigen presenting cells *in vivo*. Alternatively, antigen-presenting cells are loaded with JPL polypeptides or a nucleic acid encoding JPL polypeptides *ex vivo* and then introduced into a patient to elicit an immune response against JPL polypeptide. In another alternative, the cells presenting JPL antigen are used to stimulate the expansion of anti-

JPL polypeptide cytotoxic T lymphocytes (CTL) *ex vivo* followed by introduction of the stimulated CTL into a patient. (U.S. Patent No. 6,306,388)

Combining this type of therapy with other types of therapeutic agents or treatments such as chemotherapy or radiation is also contemplated.

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4.8.10.3 ANTI-JPL POLYPEPTIDE ANTIBODIES

Alternatively, immunotargeting involves the administration of components of the immune system, such as antibodies, antibody fragments, or primed cells of the immune system against the target. Methods of immunotargeting cancer cells using antibodies or
10 antibody fragments are well known in the art. U.S. Patent No. 6,306,393 describes the use of anti-CD22 antibodies in the immunotherapy of B-cell malignancies, and U.S. Patent No. 6,329,503 describes immunotargeting of cells that express serpentine transmembrane antigens (both U.S. patents are herein incorporated by refernce in their entirety).

15 JPL polypeptide antibodies (including humanized or human monoclonal antibodies or fragments or other modifications thereof, optionally conjugated to cytotoxic agents) may be introduced into a patient such that the antibody binds to JPL polypeptides expressed by cancer cells and mediates the destruction of the cells and the tumor and/or inhibits the growth of the cells or the tumor. Without intending to limit the disclosure,
20 mechanisms by which such antibodies can exert a therapeutic effect may include complement-mediated cytolysis, antibody-dependent cellular cytotoxicity (ADCC), modulating the physiologic function of JPL polypeptides, inhibiting binding or signal transduction pathways, modulating tumor cell differentiation, altering tumor angiogenesis factor profiles, modulating the secretion of immune stimulating or tumor suppressing
25 cytokines and growth factors, modulating cellular adhesion, and/or by inducing apoptosis. JPL polypeptide antibodies conjugated to toxic or therapeutic agents, such as radioligands or cytosolic toxins, may also be used therapeutically to deliver the toxic or therapeutic agent directly to JPL polypeptide-bearing tumor cells.

JPL polypeptide antibodies may be used to suppress the immune system in
30 patients receiving organ transplants or in patients with autoimmune diseases such as

arthritis. Healthy immune cells would be targeted by these antibodies leading their death and clearance from the system, thus suppressing the immune system.

JPL polypeptide antibodies may be used as antibody therapy for solid tumors which express this action. Cancer immunotherapy using antibodies provides a novel approach to treating cancers associated with cells that specifically express JPL polypeptides. As described above, JPL mRNA is highly expressed in melanoma tumors and in tissue adjacent to the melanoma tumors. These findings demonstrate JPL mRNA expression in tumors indicate that JPL polypeptides may be used as immunotherapeutic antibody targets and a diagnostic marker for certain cell types or disorders. Cancer immunotherapy using antibodies has been previously described for other types of cancer, including but not limited to colon cancer (Arlen *et al., Crit. Rev. Immunol.* 18:133-138 (1998)), multiple myeloma (Ozaki *et al., Blood* 90:3179-3186 (1997); Tsunenari *et al., Blood* 90:2437-2444 (1997)), gastric cancer (Kasprzyk *et al., Cancer Res.* 52:2771-2776 (1992)), B cell lymphoma (Funakoshi *et al., J. Immunother. Emphasisi Tumor Immunol.* 19:93-101 (1996)), leukemia (Zhong *et al., Leuk. Res.* 20:581-589 (1996)), colorectal cancer (Moun *et al., Cancer Res.* 54:6160-6166 (1994); Velders *et al., Cancer Res.* 55:4398-4403 (1995)), and breast cancer (Shepard *et al., J. Clin. Immunol.* 11:117-127 (1991), all of the above listed references are herein incorporated by reference in their entirety).

The melanomas which may be diagnosed and treated using the JPL polynucleotides and polypeptides comprise melanomas, metastatic melanomas, melanomas derived from either melanocytes or melanocytes related nevus cells, melanocarcinomas, melanoepitheliomas, melanosarcomas, melanoma in situ, superficial spreading melanoma, nodular melanoma, lentigo maligna melanoma, acral lentiginous melanoma, invasive melanoma or familial atypical mole and melanoma (FAM-M) syndrome. Such melanomas in mammals may be caused by, chromosomal abnormalities, degenerative growth and developmental disorders, mitogenic agents, ultraviolet radiation (UV), viral infections, inappropriate tissue expression of a gene, alterations in expression of a gene, or carcinogenic agents. The aforementioned melanomas can be diagnosed, assessed or treated by methods described in the present application.

Although JPL polypeptide antibody therapy may be useful for all stages of melanoma and the foregoing cancers, antibody therapy may be particularly appropriate in advanced or metastatic cancers. Combining the antibody therapy method with a chemotherapeutic, radiation or surgical regimen may be preferred in patients that have not received chemotherapeutic treatment, whereas treatment with the antibody therapy may be indicated for patients who have received one or more chemotherapies. Additionally, antibody therapy can also enable the use of reduced dosages of concomitant chemotherapy, particularly in patients that do not tolerate the toxicity of the chemotherapeutic agent very well. Furthermore, treatment of cancer patients with JPL polypeptide antibody with tumors resistant to chemotherapeutic agents might induce sensitivity and responsiveness to these agents in combination.

Prior to anti-JPL polypeptide immunotargeting, a patient may be evaluated for the presence and level of JPL polypeptide expression by the cancer cells, preferably using immunohistochemical assessments of tumor tissue, quantitative JPL polypeptide imaging, quantitative RT-PCR, or other techniques capable of reliably indicating the presence and degree of JPL-polypepitde expression. For example, a blood or biopsy sample may be evaluated by immunohistochemical methods to determine the presence of JPL polypeptide-expressing cells or to determine the extent of JPL polypeptide expression on the surface of the cells within the sample. Methods for immunohistochemical analysis of tumor tissues or released fragments of JPL polypeptide in the serum are well known in the art.

Anti-JPL polypeptide antibodies useful in treating melanoma and other cancers include those, which are capable of initiating a potent immune response against the tumor and those, which are capable of direct cytotoxicity. In this regard, anti-JPL polypeptide mAbs may elicit tumor cell lysis by either complement-mediated or ADCC mechanisms, both of which require an intact Fc portion of the immunoglobulin molecule for interaction with effector cell Fc receptor sites or complement proteins. In addition, anti-JPL polypeptide antibodies that exert a direct biological effect on tumor growth are useful in the practice of the invention. Potential mechanisms by which such directly cytotoxic antibodies may act include inhibition of cell growth, modulation of cellular differentiation, modulation of tumor angiogenesis factor profiles, and the induction of

apoptosis. The mechanism by which a particular anti-JPL polypeptide antibody exerts an anti-tumor effect may be evaluated using any number of *in vitro* assays designed to determine ADCC, ADMMC, complement-mediated cell lysis, and so forth, as is generally known in the art.

The anti-tumor activity of a particular anti-JPL polypeptide antibody, or combination of anti-JPL polypeptide antibody, may be evaluated *in vivo* using a suitable animal model. For example, xenogenic lymphoma cancer models wherein human lymphoma cells are introduced into immune compromised animals, such as nude or SCID mice. Efficacy may be predicted using assays, which measure inhibition of tumor formation, tumor regression or metastasis, and the like.

It should be noted that the use of murine or other non-human monoclonal antibodies, human/mouse chimeric mAbs may induce moderate to strong immune responses in some patients. In the most severe cases, such an immune response may lead to the extensive formation of immune complexes, which, potentially, can cause renal failure. Accordingly, preferred monoclonal antibodies used in the practice of the therapeutic methods of the invention are those which are either fully human or humanized and which bind specifically to the target JPL polypeptide antigen with high affinity but exhibit low or no antigenicity in the patient.

The method of the invention contemplates the administration of single anti-JPL polypeptide monoclonal antibodies (mAbs) as well as combinations, or "cocktails", of different mAbs. Two or more monoclonal antibodies that bind to JPL polypeptides may provide an improved effect compared to a single antibody. Alternatively, a combination of an anti-JPL polypeptide antibody with an antibody that binds a different antigen may provide an improved effect compared to a single antibody. Such mAb cocktails may have certain advantages inasmuch as they contain mAbs, which exploit different effector mechanisms or combine directly cytotoxic mAbs with mAbs that rely on immune effector functionality. Such mAbs in combination may exhibit synergistic therapeutic effects. In addition, the administration of anti-JPL polypeptide mAbs may be combined with other therapeutic agents, including but not limited to various chemotherapeutic agents, androgen-blockers, and immune modulators (e.g., IL-2, GM-CSF). The anti-JPL polypeptide mAbs may be administered in their "naked" or unconjugated form, or may

have therapeutic agents conjugated to them. Additionally, bispecific antibodies may be used. Such an antibody would have one antigenic binding domain specific for JPL polypeptide and the other antigenic binding domain specific for another antigen (such as CD20 for example). Finally, Fab JPL polypeptide antibodies or fragments of these antibodies (including fragments conjugated to other protein sequences or toxins) may also be used as therapeutic agents.

A. ANTI JPL POLYPETIDE ANTIBODIES

Antibodies that specifically bind JPL polypeptides are useful in compositions and methods for immunotargeting cells expressing JPL polypeptides and for diagnosing a disease or disorder wherein cells involved in the disorder express JPL polypeptides. Such antibodies include monoclonal and polyclonal antibodies, single chain antibodies, chimeric antibodies, bifunctional/bispecific antibodies, humanized antibodies, human antibodies, and complementary determining region (CDR)-grafted antibodies, including compounds that include CDR and/or antigen-binding sequences, which specifically recognize JPL polypeptides. Antibody fragments, including Fab, Fab', $F(ab')_2$, and F_v, are also useful.

The term "specific for" indicates that the variable regions of the antibodies recognize and bind JPL polypeptides exclusively (*i.e.*, able to distinguish JPL polypeptides from other similar polypeptides despite sequence identity, homology, or similarity found in the family of polypeptides), but may also interact with other proteins (for example, *S. aureus* protein A or other antibodies in ELISA techniques) through interactions with sequences outside the variable region of the antibodies, and in particular, in the constant region of the molecule. Screening assays in which one can determine binding specificity of an anti- JPL polypeptide antibody are well known and routinely practiced in the art. (Chapter 6, *Antibodies A Laboratory Manual*, Eds. Harlow, *et al.*, Cold Spring Harbor Laboratory; Cold Spring Harbor, NY (1988), herein incorporated by reference in its entirety).

JPL polypeptides can be used to immunize animals to obtain polyclonal and monoclonal antibodies that specifically react with JPL polypeptides. Such antibodies can be obtained using either the entire protein or fragments thereof as an immunogen. The

peptide immunogens additionally may contain a cysteine residue at the carboxyl terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Methods for synthesizing such peptides have been previously described (Merrifield, *J. Amer. Chem. Soc.* 85, 2149-2154 (1963); Krstenansky, *et al., FEBS Lett.* 211: 10 (1987), herein incorporated by reference in their entirety).

Techniques for preparing polyclonal and monoclonal antibodies as well as hybridomas capable of producing the desired antibody have also been previously disclosed (Campbell, *Monoclonal Antibodies Technology: Laboratory Techniques in Biochemistry and Molecular Biology*, Elsevier Science Publishers, Amsterdam, The Netherlands (1984); St. Groth, *et al., J. Immunol.* 35:1-21 (1990); Kohler and Milstein, *Nature* 256:495-497 (1975)), the trioma technique, the human B-cell hybridoma technique (Kozbor, *et al., Immunology Today* 4:72 (1983); Cole, *et al.,* in, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96 (1985) , herein incorporated by reference in their entirety).

Any animal capable of producing antibodies can be immunized with a JPL peptide or polypeptide. Methods for immunization include subcutaneous or intraperitoneal injection of the polypeptide. The amount of the JPL peptide or polypeptide used for immunization depends on the animal that is immunized, antigenicity of the peptide and the site of injection. The JPL peptide or polypeptide used as an immunogen may be modified or administered in an adjuvant in order to increase the protein's antigenicity. Methods of increasing the antigenicity of a protein are well known in the art and include, but are not limited to, coupling the antigen with a heterologous protein (such as globulin or β-galactosidase) or through the inclusion of an adjuvant during immunization.

For monoclonal antibodies, spleen cells from the immunized animals are removed, fused with myeloma cells, such as SP2/0-Ag14 myeloma cells, and allowed to become monoclonal antibody producing hybridoma cells. Any one of a number of methods well known in the art can be used to identify the hybridoma cell that produces an antibody with the desired characteristics. These include screening the hybridomas with an ELISA assay, Western blot analysis, or radioimmunoassay (Lutz, *et al., Exp. Cell Res.* 175:109-124 (1988) , herein incorporated by reference in its entirety). Hybridomas

secreting the desired antibodies are cloned and the class and subclass is determined using procedures known in the art (Campbell, A.M., *Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology*, Elsevier Science Publishers, Amsterdam, The Netherlands (1984), herein incorporated by reference in its entirety). Techniques described for the production of single chain antibodies can be adapted to produce single chain antibodies to JPL polypeptides (U.S. Patent 4,946,778, herein incorporated by reference in entirety).

For polyclonal antibodies, antibody-containing antiserum is isolated from the immunized animal and is screened for the presence of antibodies with the desired specificity using one of the above-described procedures.

Because antibodies from rodents tend to elicit strong immune responses against the antibodies when administered to a human, such antibodies may have limited effectiveness in therapeutic methods of the invention. Methods of producing antibodies that do not produce a strong immune response against the administered antibodies are well known in the art. For example, the anti- JPL polypeptide antibody can be a nonhuman primate antibody. Methods of making such antibodies in baboons are disclosed in PCT publication WO 91/11465 and Losman *et al.*, *Int. J. Cancer* 46:310-314 (1990), herein incorporated by reference in their entirety. In one embodiment, the anti-JPL polypeptide antibody is a humanized monoclonal antibody. Methods of producing humanized antibodies have been previously described. (U.S. Patent Nos. 5,997,867 and 5,985,279, Jones *et al.*, *Nature* 321:522 (1986); Riechmann *et al.*, *Nature* 332:323(1988); Verhoeyen *et al.*, *Science* 239:1534-1536 (1988); Carter *et al.*, *Proc. Nat'l Acad. Sci. USA* 89:4285-4289 (1992); Sandhu, *Crit. Rev. Biotech.* 12:437-462 (1992); and Singer, *et al.*, *J. Immun.* 150:2844-2857 (1993), herein incorporated by reference in their entirety). In another embodiment, the anti- JPL polypeptide antibody is a human monoclonal antibody. Humanized antibodies are produced by transgenic mice that have been engineered to produce human antibodies. Hybridomas derived from such mice will secrete large amounts of human monoclonal antibodies. Methods for obtaining human antibodies from transgenic mice are described in Green , *et al.*, *Nature Genet.* 7:13-21(1994), Lonberg, *et al.*, *Nature* 368:856 (1994), and Taylor, *et al.*, *Int. Immun.* 6:579 (1994), herein incorporated by reference in their entirety).

The present invention also includes the use of anti- JPL polypeptide antibody fragments. Antibody fragments can be prepared by proteolytic hydrolysis of an antibody or by expression in *E. coli* of the DNA coding for the fragment. Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies. For example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted $F(ab')_2$. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab fragments and an Fc fragment directly. These methods have been previously described (U.S. Patent Nos. 4,036,945 and 4,331,647, Nisonoff, *et al., Arch Biochem. Biophys.* 89:230 (1960); Porter, *Biochem. J.* 73:119 (1959), Edelman, *et al., Meth. Enzymol.* 1:422 (1967), herein incorporated by reference in their entirety). Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody. For example, Fv fragments comprise an association of V_H and V_L chains, which can be noncovalent (Inbar *et al., Proc. Nat'l Acad. Sci. USA* 69:2659 (1972), herein incorporated by reference in its entirety). Alternatively, the variable chains can be linked by an intermolecular disulfide bond or cross-linked by chemicals such as glutaraldehyde.

In one embodiment, the Fv fragments comprise V_H and V_L chains that are connected by a peptide linker. These single-chain antigen binding proteins (sFv) are prepared by constructing a structural gene comprising DNA sequences encoding the V_H and V_L domains which are connected by an oligonucleotide. The structural gene is inserted into an expression vector, which is subsequently introduced into a host cell, such as *E. coli.* The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing sFvs have been previously described (U.S. Patent No. 4,946,778, Whitlow, *et al., Methods: A Companion to Methods in Enzymology* 2:97 (1991), Bird, *et al., Science* 242:423 (1988), Pack, *et al., Bio/Technology* 11:1271 (1993), herein incorporated by reference in their entirety).

Another form of an antibody fragment is a peptide coding for a single complementarity-determining region (CDR). CDR peptides ("minimal recognition units") can be obtained by constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthesize the variable region from RNA of antibody-producing cells (Larrick, *et al.*, *Methods: A Companion to Methods in Enymology* 2:106 (1991); Courtenay-Luck, pp. 166-179 in, *Monoclonal Antibodies Production, Engineering and Clinical Applications*, Eds. Ritter *et al.*, Cambridge University Press (1995); Ward, *et al.*, pp. 137-185 in, *Monoclonal Antibodies Principles and Applications*, Eds. Birch *et al.*, Wiley-Liss, Inc. (1995), herein incorporated by reference in their entirety).

The present invention further provides the above- described antibodies in detectably labeled form. Antibodies can be detectably labeled through the use of radioisotopes, affinity labels (such as biotin, avidin, etc.), enzymatic labels (such as horseradish peroxidase, alkaline phosphatase, etc.) fluorescent labels (such as FITC or rhodamine, etc.), paramagnetic atoms, etc. Procedures for accomplishing such labeling have been previously disclosed (Sternberger, *et al., J. Histochem. Cytochem.* 18:315 (1970); Bayer, *et al., Meth. Enzym.* 62:308 (1979); Engval, *et al., Immunol.* 109:129 (1972); Goding, *J. Immunol. Meth.* 13:215 (1976), herein incorporated by reference in their entirety).

The labeled antibodies can be used for *in vitro*, *in vivo*, and *in situ* assays to identify cells or tissues in which JPL polypeptides are expressed. Furthermore, the labeled antibodies can be used to identify the presence of secreted JPL polypeptides in a biological sample, such as a blood, urine, saliva samples.

B. ANTI -JPL POLYPEPTIDE ANTIBODY CONJUGATES

The present invention contemplates the use of "naked" anti- JPL polypeptide antibodies, as well as the use of immunoconjugates. Immnunoconjugates can be prepared by indirectly conjugating a therapeutic agent such as a cytotoxic agent to an antibody component. Toxic moieties include, for example, plant toxins, such as abrin, ricin, modeccin, viscumin, pokeweed anti-viral protein, saporin, gelonin, momoridin, trichosanthin, barley toxin; bacterial toxins, such as *Diptheria* toxin, *Pseudomonas*

endotoxin and exotoxin, *Staphylococcal* enterotoxin A; fungal toxins, such as α-sarcin, restrictocin; cytotoxic RNases, such as extracellular pancreatic RNases; DNase I (Pastan, *et al., Cell* 47:641 (1986); Goldenberg, *Cancer Journal for Clinicians* 44:43 (1994)), calicheamicin, and radioisotopes, such as ^{32}P, ^{67}Cu, ^{77}As, ^{105}Rh, ^{109}Pd, ^{111}Ag, ^{121}Sn, ^{131}I, ^{166}Ho, ^{177}Lu, ^{186}Re, ^{188}Re, ^{194}Ir, ^{199}Au (Illidge, T.M. & Brock, S. 2000, Curr Pharm. Design 6: 1399, herein incorporated by reference in its entirety). In humans, clinical trials are underway utilizing a yttrium-90 conjugated anti-CD20 antibody for B cell lymphomas (*Cancer Chemother Pharmacol* 48(Suppl 1):S91-S95 (2001), herein incorporated by reference in its entirety).

General techniques have been previously described (U.S. Patent Nos. 6,306,393 and 5,057,313, Shih, *et al., Int. J. Cancer* 41:832-839 (1988); Shih, *et al., Int. J. Cancer* 46:1101-1106 (1990)). The general method involves reacting an antibody component having an oxidized carbohydrate portion with a carrier polymer that has at least one free amine function and that is loaded with a plurality of drug, toxin, chelator, boron addends, or other therapeutic agent. This reaction results in an initial Schiff base (imine) linkage, which can be stabilized by reduction to a secondary amine to form the final conjugate.

The carrier polymer is preferably an aminodextran or polypeptide of at least 50 amino acid residues, although other substantially equivalent polymer carriers can also be used. Preferably, the final immunoconjugate is soluble in an aqueous solution, such as mammalian serum, for ease of administration and effective targeting for use in therapy. Thus, solubilizing functions on the carrier polymer will enhance the serum solubility of the final immunoconjugate. In particular, an aminodextran will be preferred.

The process for preparing an inmmunoconjugate with an aminodextran carrier typically begins with a dextran polymer, advantageously a dextran of average molecular weight of about 10,000-100,000. The dextran is reacted with an oxidizing agent to affect a controlled oxidation of a portion of its carbohydrate rings to generate aldehyde groups. The oxidation is conveniently effected with glycolytic chemical reagents such as $NaIO_4$, according to conventional procedures. The oxidized dextran is then reacted with a polyamine, preferably a diamine, and more preferably, a mono- or polyhydroxy diamine. Suitable amines include ethylene diamine, propylene diamine, or other like polymethylene diamines, diethylene triamine or like polyamines, 1,3-diamino-2-

hydroxypropane, or other like hydroxylated diamines or polyamines, and the like. An excess of the amine relative to the aldehyde groups of the dextran is used to ensure substantially complete conversion of the aldehyde functions to Schiff base groups. A reducing agent, such as $NaBH_4$, $NaBH_3$ CN or the like, is used to effect reductive stabilization of the resultant Schiff base intermediate. The resultant adduct can be purified by passage through a conventional sizing column or ultrafiltration membrane to remove cross-linked dextrans. Other conventional methods of derivatizing a dextran to introduce amine functions can also be used, e.g., reaction with cyanogen bromide, followed by reaction with a diamine.

The amninodextran is then reacted with a derivative of the particular drug, toxin, chelator, immunomodulator, boron addend, or other therapeutic agent to be loaded, in an activated form, preferably, a carboxyl-activated derivative, prepared by conventional means, *e.g.*, using dicyclohexylcarbodiimide (DCC) or a water soluble variant thereof, to form an intermediate adduct. Alternatively, polypeptide toxins such as pokeweed antiviral protein or ricin A-chain, and the like, can be coupled to aminodextran by glutaraldehyde condensation or by reaction of activated carboxyl groups on the protein with amines on the aminodextran.

Chelators for radiometals or magnetic resonance enhancers are well-known in the art. Typical are derivatives of ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA). These chelators typically have groups on the side chain by which the chelator can be attached to a carrier. Such groups include, *e.g.*, benzylisothiocyanate, by which the DTPA or EDTA can be coupled to the amine group of a carrier. Alternatively, carboxyl groups or amine groups on a chelator can be coupled to a carrier by activation or prior derivatization and then coupling, all by well-known means.

Boron addends, such as carboranes, can be attached to antibody components by conventional methods. For example, carboranes can be prepared with carboxyl functions on pendant side chains, as is well known in the art. Attachment of such carboranes to a carrier, e.g., aminodextran, can be achieved by activation of the carboxyl groups of the carboranes and condensation with amines on the carrier to produce an intermediate

conjugate. Such intermediate conjugates are then attached to antibody components to produce therapeutically useful immunoconjugates, as described below.

A polypeptide carrier can be used instead of aminodextran, but the polypeptide carrier should have at least 50 amino acid residues in the chain, preferably 100-5000 amino acid residues. At least some of the amino acids should be lysine residues or glutamate or aspartate residues. The pendant amines of lysine residues and pendant carboxylates of glutamine and aspartate are convenient for attaching a drug, toxin, immunomodulator, chelator, boron addend or other therapeutic agent. Examples of suitable polypeptide carriers include polylysine, polyglutamic acid, polyaspartic acid, co-polymers thereof, and mixed polymers of these amino acids and others, e.g., serines, to confer desirable solubility properties on the resultant loaded carrier and immunoconjugate.

Conjugation of the intermediate conjugate with the antibody component is effected by oxidizing the carbohydrate portion of the antibody component and reacting the resulting aldehyde (and ketone) carbonyls with amine groups remaining on the carrier after loading with a drug, toxin, chelator, immunomodulator, boron addend, or other therapeutic agent. Alternatively, an intermediate conjugate can be attached to an oxidized antibody component via amine groups that have been introduced in the intermediate conjugate after loading with the therapeutic agent. Oxidation is conveniently effected either chemically, e.g., with $NaIO_4$ or other glycolytic reagent, or enzymatically, *e.g.*, with neuraminidase and galactose oxidase. In the case of an aminodextran carrier, not all of the amines of the aminodextran are typically used for loading a therapeutic agent. The remaining amines of aminodextran condense with the oxidized antibody component to form Schiff base adducts, which are then reductively stabilized, normally with a borohydride reducing agent.

Analogous procedures are used to produce other immunoconjugates according to the invention. Loaded polypeptide carriers preferably have free lysine residues remaining for condensation with the oxidized carbohydrate portion of an antibody component. Carboxyls on the polypeptide carrier can, if necessary, be converted to amines by, e.g., activation with DCC and reaction with an excess of a diamine.

The final immunoconjugate is purified using conventional techniques, such as sizing chromatography on Sephacryl S-300 or affinity chromatography using one or more JPL polypeptide epitopes.

Alternatively, immunoconjugates can be prepared by directly conjugating an antibody component with a therapeutic agent. The general procedure is analogous to the indirect method of conjugation except that a therapeutic agent is directly attached to an oxidized antibody component. It will be appreciated that other therapeutic agents can be substituted for the chelators described herein. Those of skill in the art will be able to devise conjugation schemes without undue experimentation.

As a further illustration, a therapeutic agent can be attached at the hinge region of a reduced antibody component via disulfide bond formation. For example, the tetanus toxoid peptides can be constructed with a single cysteine residue that is used to attach the peptide to an antibody component. As an alternative, such peptides can be attached to the antibody component using a heterobifunctional cross-linker, such as N-succinyl 3-(2-pyridyldithio)proprionate (SPDP) (Yu, *et al., Int. J. Cancer* 56:244 (1994)). General techniques for such conjugation have been previously described (Wong, *Chemistry of Protein Conjugation and Cross-linking*, CRC Press (1991); Upeslacis, *et al.*, pp. 187-230 in, *Monoclonal Antibodies Principles and Applications*, Eds. Birch *et al.*, Wiley-Liss, Inc. (1995); Price, pp. 60-84 in, *Monoclonal Antibodies: Production, Engineering and Clinical Applications* Eds. Ritter, *et al.*, Cambridge University Press (1995), herein incorporated by reference in their entirety).

As described above, carbohydrate moieties in the Fc region of an antibody can be used to conjugate a therapeutic agent. However, the Fc region may be absent if an antibody fragment is used as the antibody component of the immunoconjugate. Nevertheless, it is possible to introduce a carbohydrate moiety into the light chain variable region of an antibody or antibody fragment (Leung, *et al., J. Immunol.* 154:5919-5926 (1995); U.S. Pat. No. 5,443,953, herein incorporated by reference in their entirety). The engineered carbohydrate moiety is then used to attach a therapeutic agent.

In addition, those of skill in the art will recognize numerous possible variations of the conjugation methods. For example, the carbohydrate moiety can be used to attach polyethyleneglycol in order to extend the half-life of an intact antibody, or antigen-

binding fragment thereof, in blood, lymph, or other extracellular fluids. Moreover, it is possible to construct a "divalent immunoconjugate" by attaching therapeutic agents to a carbohydrate moiety and to a free sulfhydryl group. Such a free sulfhydryl group may be located in the hinge region of the antibody component.

C. ANTI-JPL POLYPEPTIDE ANTIBODY FUSION PROTEINS

When the therapeutic agent to be conjugated to the antibody is a protein, the present invention contemplates the use of fusion proteins comprising one or more anti-JPL polypeptide antibody moieties and an immunomodulator or toxin moiety. Methods of making antibody fusion proteins have been previously described (U.S. Patent No. 6,306,393, herein incorporated by reference in its entirety). Antibody fusion proteins comprising an interleukin-2 moiety have also been previously disclosed (Boleti, *et al., Ann. Oncol.* 6:945 (1995), Nicolet, *et al., Cancer Gene Ther.* 2:161 (1995), Becker, *et al., Proc. Nat'l Acad. Sci. USA* 93:7826 (1996), Hank, *et al., Clin. Cancer Res.* 2:1951 (1996), Hu, *et al., Cancer Res.* 56:4998 (1996), herein incorporated by reference in their entirety). In addition, Yang, *et al., Hum. Antibodies Hybridomas* 6:129 (1995), describe a fusion protein that includes an $F(ab')_2$ fragment and a tumor necrosis factor alpha moiety.

Methods of making antibody-toxin fusion proteins in which a recombinant molecule comprises one or more antibody components and a toxin or chemotherapeutic agent also are known to those of skill in the art. For example, antibody-*Pseudomonas* exotoxin A fusion proteins have been described (Chaudhary, *et al., Nature* 339:394 (1989), Brinkmann, *et al., Proc. Nat'l Acad. Sci. USA* 88:8616 (1991), Batra, *et al., Proc. Natl. Acad. Sci. USA* 89:5867 (1992), Friedman, *et al., J. Immunol.* 150:3054 (1993), Wels, *et al., Int. J. Can.* 60:137 (1995), Fominaya *et al., J. Biol. Chem.* 271:10560 (1996), Kuan, *et al., Biochemistry* 35:2872 (1996), Schmidt, *et al., Int. J. Can.* 65:538 (1996), herein incorporated by reference in their entirety). Antibody-toxin fusion proteins containing a diphtheria toxin moiety have been described (Kreitman, *et al., Leukemia* 7:553 (1993), Nicholls, *et al., J. Biol. Chem.* 268:5302 (1993), Thompson, *et al., J. Biol. Chem.* 270:28037 (1995), and Vallera, *et al., Blood* 88:2342 (1996). Deonarain *et al.* (*Tumor Targeting* 1:177 (1995), herein incorporated by reference in their entirety) have described an antibody-toxin fusion protein having an RNase moiety, while

Linardou, *et al.* (*Cell Biophys.* 24-25:243 (1994)), produced an antibody-toxin fusion protein comprising a DNase I component. Gelonin and *Staphylococcal* enterotoxin-A have been used as the toxin moieties in antibody-toxin fusion proteins (Wang, *et al.*, Abstracts of the 209th ACS National Meeting, Anaheim, Calif., Apr. 2-6, 1995, Part 1, BIOT005; Dohlsten, *et al., Proc. Nat'l Acad. Sci. USA* 91:8945 (1994), herein incorporated by reference in their entirety).

D. PEPTIDES

Peptides of the cell surface antigens of the invention themselves, such as fragments of the extracellular region, may be used to target toxins or radioisotopes to tumor cells *in vivo* by binding to or interacting with the cell surface antigens of the invention expressed on tumor or diseased cells. Much like an antibody, these fragments may specifically target cells expressing this antigen. Targeted delivery of these cytotoxic agents to the tumor cells would result in cell death and suppression of tumor growth. An example of the ability of an extracellular fragment binding to and activating its intact receptor (by homophilic binding) has been demonstrated with the CD84 receptor (Martin *et al., J. Immunol.* 167:3668-3676 (2001), herein incorporated by reference in its entirety).

Extracellular fragments of the cell surface antigens of the invention may also be used to modulate immune cells expressing the protein. Extracellular domain fragments of the cell surface antigen may bind to and activate its own receptor on the cell surface, which may result in stimulating the release of cytokines (such as interferon gamma from NK cells, T cells, B cells or myeloid cells, for example) that may enhance or suppress the immune system. Additionally, binding of these fragments to cells bearing cell surface antigens of the invention may result in the activation of these cells and also may stimulate proliferation. Some fragments may bind to the intact cell surface antigen of the invention and block activation signals and cytokine release by immune cells. These fragments would then have an immunosuppressive effect. Fragments that activate and stimulate the immune system may have anti-tumor properties. These fragments may stimulate an immunological response that can result in immune-mediated tumor cell killing. The same fragments may result in stimulating the immune system to mount an enhanced

response to foreign invaders such as viruses and bacteria. Fragments that suppress the immune response may be useful in treating lymphoproliferative disorders, auto-immune diseases, graft-vs-host disease, and inflammatory diseases, such as emphysema.

E. OTHER BINDING PEPTIDES OR SMALL MOLECULES

Screening of organic compound or peptide libraries with recombinantly expressed JPL protein of the invention may be useful for identification of therapeutic molecules that function to specifically bind to or even inhibit the activity of JPL proteins. Synthetic and naturally occurring products can be screened in a number of ways deemed routine to those of skill in the art. Random peptide libraries are displayed on phage (phage display) or on bacteria, such as on *E. coli*. These random peptide display libraries can be used to screen for peptides which interact with a known target which can be a protein or a polypeptide, such as a ligand or receptor, a biological or synthetic macromolecule, or organic or inorganic substances. By way of example, diversity libraries, such as random or combinatorial peptide or nonpeptide libraries can be screened for molecules that specifically bind to JPL polypeptides. Many libraries are known in the art that can be used, *i.e.* chemically synthesized libraries, recombinant (*i.e.* phage display libraries), and in vitro translation-based libraries. Techniques for creating and screening such random peptide display libraries are known in the art (Ladner *et al.*, U.S. Patent No. 5,223, 409; Ladner *et al.*, U.S. Patent No. 4,946,778; Ladner *et al.*, U.S. Patent No. 5,403,484; Ladner *et al.*, U.S. Patent No. 5,571,698, all of which are herein incorporated by reference in their entirety) and random peptide display libraries and kits for screening such libraries are available commercially, for instance from Clontech (Palo Alto, CA), Invitrogen Inc. (San Diego, CA), New England Biolabs, Inc. (Beverly, MA), and Pharmacia KLB Biotechnology Inc. (Piscataway, NJ). Random peptide display libraries can be screened using the JPL sequences disclosed herein to identify proteins which bind to the JPL of the invention.

Examples of chemically synthesized libraries are described in Fodor *et al.*, *Science* 251:767-773 (1991); Houghten *et al.*, *Nature* 354:84-86 (1991); Lam *et al.*, *Nature* 354:82-84 (1991); Medynski, *Bio/Technology* 12:709-710 (1994); Gallop *et al.*, *J. Med. Chem.* 37:1233-1251 (1994); Ohlmeyer *et al.*, *Proc. Natl. Acad. Sci. USA*

90:10922-10926 (1993); Erb *et al., Proc. Natl. Acad. Sci. USA* 91:11422-11426 (1994); Houghten *et al., Biotechniques* 13:412 (1992); Jayawickreme *et al., Proc. Natl. Acad. Sci. USA* 91:1614-1618 (1994); Salmon *et al., Proc. Natl. Acad. Sci. USA* 90:11708-11712 (1993); PCT Publication No. WO 93/20242; Brenner and Lerner, *Proc. Natl. Acad. Sci. USA* 89:5381-5383 (1992), all of which are herein incorporated by reference in their entirety.

Examples of phage display libraries are described in Scott and Smith, *Science* 249:386-390 (1990); Devlin *et al., Science* 249:404-406 (1990); Christian *et al., J. Mol. Biol.* 227:711-718 (1992); Lenstra, *J. Immunol Meth.* 152:149-157 (1992); Kay *et al., Gene* 128:59-65 (1993); PCT Publication No. WO 94/18318, all of which are herein incorporated by reference in their entirety.

In vitro translation-based libraries include but are not limited to those described in PCT Publication No. WO 91/05058, and Mattheakis *et al., Proc. Natl. Acad. Sci. USA* 91:9022-9026 (1994), both of which are herein incorporated by reference in their entirety.

By way of examples of nonpeptide libraries, a benzodiazepine library (see for example, Bunin *et al., Proc. Natl. Acad. Sci. USA* 91:4708-4712 (1994), herein incorporated by reference in its entirety) can be adapted for use. Peptoid libraries (Simon *et al., Proc. Natl. Acad. Sci. USA* 89:9367-9371 (1992), herein incorporated by reference in its entirety) can also be used. Another example of a library that can be used, in which the amide functionalities in peptides have been permethylated to generate a chemically transformed combinatorial library, is described by Ostresh *et al.* (*Proc. Natl. Acad. Sci. USA* 91:11138-11142 (1994), herein incorporated by reference in its entirety).

Screening the libraries can be accomplished by any of a variety of commonly known methods. See, for example, the following references which disclose screening of peptide libraries: Parmley and Smith, *Adv. Exp. Med. Biol.* 251:215-218 (1989); Scott and Smith, *Science* 249:386-390 (1990); Fowlkes *et al., Biotechniques* 13:422-427 (1992); Oldenburg *et al., Proc. Natl. Acad. Sci. USA* 89:5393-5397 (1992); Yu *et al., Cell* 76:933-945 (1994); Staudt *et al., Science* 241:577-580 (1988); Bock *et al., Nature* 355:564-566 (1992); Tuerk *et al., Proc. Natl. Acad. Sci. USA* 89:6988-6992 (1992); Ellington *et al., Nature* 355:850-852 (1992); Rebar and Pabo, *Science* 263:671-673

(1993); and PCT Publication No. WO 94/18318, all of which are herein incorporated by reference in their entirety.

In a specific embodiment, screening can be carried out by contacting the library members with a JPL protein (or nucleic acid or derivative) immobilized on a solid phase and harvesting those library members that bind to the protein (or nucleic acid or derivative). Examples of such screening methods, termed "panning" techniques are described by way of example in Parmley and Smith, *Gene* 73:305-318 (1988); Fowlkes *et al., Biotechniques* 13:422-427 (1992); PCT Publication No. WO 94/18318, all of which are herein incorporated by reference in their entirety, and in references cited hereinabove.

In another embodiment, the two-hybrid system for selecting interacting protein in yeast (Fields and Song, *Nature* 340:245-246 (1989); Chien *et al., Proc. Natl. Acad. Sci. USA* 88:9578-9582 (1991), both of which are herein incorporated by reference in their entirety) can be used to identify molecules that specifically bind to a JPL protein or derivative.

These "binding polypeptides" or small molecules which interact with JPL polypeptides of the invention can be used for tagging or targeting cells; for isolating homolog polypeptides by affinity purification; they can be directly or indirectly conjugated to drugs, toxins, radionuclides and the like. These binding polypeptides or small molecules can also be used in analytical methods such as for screening expression libraries and neutralizing activity, *i.e.*, for blocking interaction between ligand and receptor, or viral binding to a receptor. The binding polypeptides or small molecules can also be used for diagnostic assays for determining circulating levels of JPL polypeptides of the invention; for detecting or quantitating soluble JPL polypeptides as marker of underlying pathology or disease. These binding polypeptides or small molecules can also act as JPL "antagonists" to block JPL binding and signal transduction *in vitro* and *in vivo*. These anti-JPL binding polypeptides or small molecules would be useful for inhibiting JPL activity or protein binding.

Binding polypeptides can also be directly or indirectly conjugated to drugs, toxins, radionuclides and the like, and these conjugates used for *in vivo* diagnostic or therapeutic applications. Binding peptides can also be fused to other polypeptides, for example an immunoglobulin constant chain or portions thereof, to enhance their half-life,

and can be made multivalent (through, *e.g.* branched or repeating units) to increase binding affinity for the JPL. For instance, binding polypeptides of the present invention can be used to identify or treat tissues or organs that express a corresponding anti-complementary molecule (receptor or antigen, respectively, for instance). More specifically, binding polypeptides or bioactive fragments or portions thereof, can be coupled to detectable or cytotoxic molecules and delivered to a mammal having cells, tissues or organs that express the anti-complementary molecule.

Suitable detectable molecules may be directly or indirectly attached to the binding polypeptide, and include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent markers, chemiluminescent markers, magnetic particles and the like. Suitable cytotoxic molecules may be directly or indirectly attached to the binding polypeptide, and include bacterial or plant toxins (for instance, diphtheria toxin, *Pseudomonas* exotoxin, ricin, abrin and the like), as well as therapeutic radionuclides, such as iodine-131, rhenium-188, or yttrium-90 (either directly attached to the binding polypeptide, or indirectly attached through a means of a chelating moiety, for instance). Binding polypeptides may also be conjugated to cytotoxic drugs, such as adriamycin. For indirect attachment of a detectable or cytotoxic molecule, the detectable or cytotoxic molecule can be conjugated with a member of a complementary/anticomplementary pair, where the other member is bound to the binding polypeptide. For these purposes, biotin/streptavidin is an exemplary complementary/anticomplementary pair.

In another embodiment, binding polypeptide-toxin fusion proteins can be used for targeted cell or tissue inhibition or ablation (for instance, to treat cancer cells or tissues). Alternatively, if the binding polypeptide has multiple functional domains (*i.e.*, an activation domain or a ligand binding domain, plus a targeting domain), a fusion protein including only the targeting domain may be suitable for directing a detectable molecule, a cytotoxic molecule, or a complementary molecule to a cell or tissue type of interest. In instances where the domain only fusion protein includes a complementary molecule, the anti-complementary molecule can be conjugated to a detectable or cytotoxic molecule. Such domain-complementary molecule fusion proteins thus represent a generic targeting vehicle for cell/tissue-specific delivery of generic anti-complementary-detectable/cytotoxic molecule conjugates.

4.8.10.4 DISEASES AMENABLE TO ANTI-JPL POLYPEPTIDE TARGETING

In one aspect, the present invention provides reagents and methods useful for treating diseases and conditions wherein cells associated with the disease or disorder express JPL polypeptides. These diseases can include melanoma and other cancers, and other hyperproliferative conditions, such as hyperplasia, psoriasis, contact dermatitis, immunological disorders, and infertility. Whether the cells associated with a disease or condition express JPL polypeptides can be determined using the diagnostic methods described herein.

Comparisons of JPL mRNA and protein expression levels between diseased cells, tissue or fluid (blood, lymphatic fluid, etc.) and corresponding normal samples are made to determine if the patient will be responsive to JPL targeting therapy. Methods for detecting and quantifying the expression of JPL polypeptide mRNA or protein use standard nucleic acid and protein detection and quantitation techniques that are well known in the art and are described in Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, NY (1989) or Ausubel, *et al.*, *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, NY (1989), both of which are incorporated herein by reference in their entirety. Standard methods for the detection and quantification of JPL mRNA include *in situ* hybridization using labeled JPL riboprobes (Gemou-Engesaeth, *et al.*, *Pediatrics* 109: E24-E32 (2002), herein incorporated by reference in its entirety), Northern blot and related techniques using JPL polynucleotide probes (Kunzli, *et al.*, *Cancer* 94: 228 (2002), herein incorporated by reference in its entirety , herein incorporated by reference in its entirety), RT-PCR analysis using JPL-specific primers (Angchaiskisiri, *et al.*, *Blood* 99:130 (2002)), and other amplification detection methods, such as branched chain DNA solution hybridization assay (Jardi, *et al., J. Viral Hepat.* 8:465-471 (2001), herein incorporated by reference in its entirety), transcription-mediated amplification (Kimura, *et al.*, *J. Clin. Microbiol.* 40:439-445 (2002)), microarray products, such as oligos, cDNAs, and monoclonal antibodies, and real-time PCR (Simpson, *et al.*, *Molec. Vision*, 6:178-183 (2000), herein incorporated by reference in its entirety). Standard methods for the detection and quantification of JPL protein include western blot analysis (Sambrook, *et*

al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, NY (1989), Ausubel, *et al.*, *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, NY (1989)), immunocytochemistry (Racila, *et al.*, *Proc. Natl. Acad. Sci. USA* 95:4589-4594 (1998)*supra*), and a variety of immunoassays, including enzyme-linked immunosorbant assay (ELISA), radioimmuno assay (RIA), and specific enzyme immunoassay (EIA) (Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, NY (1989), Ausubel, *et al.*, *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, NY (1989)). Peripheral blood cells can also be analyzed for JPL polypeptide expression using flow cytometry using, for example, immunomagnetic beads specific for JPL polypeptides (Racila, *et al.*, *Proc. Natl. Acad. Sci. USA* 95:4589-4594 (1998)) or biotinylated JPL polypeptides antibodies (Soltys, *et al.*, *J. Immunol.* 168:1903 (2002)). Tumor aggressiveness can be gauged by determining the levels of JPL polypeptide protein or mRNA in tumor cells compared to the corresponding normal cells (Orlandi, *et al.*, *Cancer Res.* 62:567 (2002)). In one embodiment, the disease or disorder is a cancer.

The cancers treatable by methods of the present invention preferably occur in mammals. Mammals include, for example, humans and other primates, as well as pet or companion animals such as dogs and cats, laboratory animals such as rats, mice and rabbits, and farm animals such as horses, pigs, sheep, and cattle.

Tumors or neoplasms include growths of tissue cells in which the multiplication of the cells is uncontrolled and progressive. Some such growths are benign, but others are termed "malignant" and may lead to death of the organism. Malignant neoplasms or "cancers" are distinguished from benign growths in that, in addition to exhibiting aggressive cellular proliferation, they may invade surrounding tissues and metastasize. Moreover, malignant neoplasms are characterized in that they show a greater loss of differentiation (greater "dedifferentiation"), and greater loss of their organization relative to one another and their surrounding tissues. This property is also called "anaplasia."

Neoplasms treatable by the present invention also include solid phase tumors/malignancies, *i.e.*, carcinomas, locally advanced tumors and human soft tissue sarcomas. Carcinomas include those malignant neoplasms derived from epithelial cells that infiltrate (invade) the surrounding tissues and give rise to metastastic cancers,

including lymphatic metastases. Adenocarcinomas are carcinomas derived from glandular tissue, or which form recognizable glandular structures. Another broad category or cancers includes sarcomas, which are tumors whose cells are embedded in a fibrillar or homogeneous substance like embryonic connective tissue. The invention also enables treatment of cancers of the myeloid or lymphoid systems, including leukemias, lymphomas and other cancers that typically do not present as a tumor mass, but are distributed in the vascular or lymphoreticular systems.

The type of cancer or tumor cells that may be amenable to treatment according to the invention include, for example, acute lymphocytic leukemia, acute nonlymphocytic leukemia, chronic lymphocytic leukemia, chronic myelocytic leukemia, cutaneous T-cell lymphoma, hairy cell leukemia, acute myeloid leukemia, erythroleukemia, chronic myeloid (granulocytic) leukemia, Hodgkin's disease, and non-Hodgkin's lymphoma, gastrointestinal cancers including esophageal cancer, stomach cancer, colon cancer, colorectal cancer, polyps associated with colorectal neoplasms, pancreatic cancer and gallbladder cancer, cancer of the adrenal cortex, ACTH-producing tumor, bladder cancer, brain cancer including intrinsic brain tumors, neuroblastomas, astrocytic brain tumors, gliomas, and metastatic tumor cell invasion of the central nervous system, Ewing's sarcoma, head and neck cancer including mouth cancer and larynx cancer, , kidney cancer including renal cell carcinoma, liver cancer, lung cancer including small and non-small cell lung cancers, malignant peritoneal effusion, malignant pleural effusion, skin cancers including malignant melanoma, tumor progression of human skin keratinocytes, squamous cell carcinoma, basal cell carcinoma, and hemangiopericytoma, mesothelioma, Kaposi's sarcoma, bone cancer including osteomas and sarcomas such as fibrosarcoma and osteosarcoma, cancers of the female reproductive tract including uterine cancer, endometrial cancer, ovarian cancer, ovarian (germ cell) cancer and solid tumors in the ovarian follicle, vaginal cancer, cancer of the vulva, and cervical cancer; breast cancer (small cell and ductal), penile cancer, prostate cancer, retinoblastoma, testicular cancer, thyroid cancer, trophoblastic neoplasms, and Wilms' tumor.

The invention is particularly illustrated herein in reference to treatment of certain types of experimentally defined cancers. In these illustrative treatments, standard state-of-the-art *in vitro* and *in vivo* models have been used. These methods can be used to

identify agents that can be expected to be efficacious in *in vivo* treatment regimens. However, it will be understood that the method of the invention is not limited to the treatment of these tumor types, but extends to any cancer derived from any organ system. Leukemias can result from uncontrolled B cell proliferation initially within the bone marrow before disseminating to the peripheral blood, spleen, lymph nodes and finally to other tissues. Uncontrolled B cell proliferation also may result in the development of lymphomas that arise within the lymph nodes and then spread to the blood and bone marrow. Targeting JPL polypeptides may be useful in treating B cell malignancies, leukemias, lymphomas and myelomas including but not limited to multiple myeloma, Burkitt's lymphoma, cutaneous B cell lymphoma, primary follicular cutaneous B cell lymphoma, B lineage acute lymphoblastic leukemia (ALL), B cell non-Hodgkin's lymphoma (NHL), B cell chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia, hairy cell leukemia (HCL), splenic marginal zone lymphoma, diffuse large B cell lymphoma, prolymphocytic leukemia (PLL), lymphoplasma cytoid lymphoma, mantle cell lymphoma, mucosa-associated lymphoid tissue (MALT) lymphoma, primary thyroid lymphoma, intravascular malignant lymphomatosis, splenic lymphoma, Hodgkin's Disease, intragraft angiotropic large-cell lymphoma, acute myelogenous leukemia, acute myelomonocytic leukemia, acute lymphoblastic leukemia, chronic myelogenic leukemia, malignant lymphoma, and lymphosarcoma cell leukemia. Other diseases that may be treated by the methods of the present invention include multicentric Castleman's disease, primary amyloidosis, Franklin's disease, Seligmann's disease, primary effusion lymphoma, post-transplant lymphoproliferative disease (PTLD) [associated with EBV infection.], paraneoplastic pemphigus, chronic lymphoproliferative disorders, X-linked lymphoproliferative syndrome (XLP), acquired angioedema, angioimmunoblastic lymphadenopathy with dysproteinemia, Herman's syndrome, post-splenectomy syndrome, congenital dyserythropoietic anemia type III, lymphoma-associated hemophagocytic syndrome (LAHS), necrotizing ulcerative stomatitis, Kikuchi's disease, lymphomatoid granulomatosis, Richter's syndrome, polycythemic vera (PV), Gaucher's disease, Gougerot-Sjogren syndrome, Kaposi's sarcoma, cerebral lymphoplasmocytic proliferation (Bind and Neel syndrome), X-linked lymphoproliferative disorders, pathogen associated disorders such as mononucleosis

(Epstein Barr Virus), lymphoplasma cellular disorders, post-transplantational plasma cell dyscrasias, and Good's syndrome.

Autoimmune diseases can be associated with hyperactive B cell activity that results in autoantibody production. Inhibition of the development of autoantibody-producing cells or proliferation of such cells may be therapeutically effective in decreasing the levels of autoantibodies in autoimmune diseases including but not limited to systemic lupus erythematosus, Crohn's Disease, graft-verses-host disease, Graves' disease, myasthenia gravis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, asthma, cryoglubulinemia, primary biliary sclerosis, pernicious anemia, Waldenstrom macroglobulinemia, hyperviscosity syndrome, macroglobulinemia, cold agglutinin disease, monoclonal gammopathy of undetermined origin, anetoderma and POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, M component, skin changes), connective tissue disease, multiple sclerosis, cystic fibrosis, rheumatoid arthritis, autoimmune pulmonary inflammation, psoriasis, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitis, autoimmune inflammatory eye disease, Goodpasture's disease, Rasmussen's encephalitis, dermatitis herpetiformis, thyoma, autoimmune polyglandular syndrome type 1, primary and secondary membranous nephropathy, cancer-associated retinopathy, autoimmune hepatitis type 1, mixed cryoglobulinemia with renal involvement, cystoid macular edema, endometriosis, IgM polyneuropathy (including Hyper IgM syndrome), demyelinating diseases, angiomatosis, and monoclonal gammopathy.

Targeting JPL polypeptides may also be useful in the treatment of allergic reactions and conditions *e.g.*, anaphylaxis, serum sickness, drug reactions, food allergies, insect venom allergies, mastocytosis, allergic rhinitis, hypersensitivity pneumonitis, urticaria, angioedema, eczema, atopic dermatitis, allergic contact dermatitis, erythema multiforme, Stevens-Johnson syndrome, allergic conjunctivitis, atopic keratoconjunctivitis, venereal keratoconjunctivitis, giant papillary conjunctivitis, allergic gastroenteropathy, inflammatory bowel disorder (IBD), and contact allergies, such as asthma (particularly allergic asthma), or other respiratory problems.

Targeting JPL may also be useful in the management or prevention of transplant rejection in patients in need of transplants such as stem cells, tissue or organ transplant.

Thus, one aspect of the invention may find therapeutic utility in various diseases (such as those usually treated with transplantation, including without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria) as wells in repopulating the stem cell compartment post irridiation/chemotherapy, either in-vivo or ex-vivo (i.e. in conjunction with bone marrow transplantation or with peripheral progenitor cell transplantation (homologous or heterologous) as normal cells or genetically manipulated for gene therapy.

Targeting JPL may also be possible to modulate immune responses, in a number of ways. Down regulation may be in the form of inhibiting or blocking an immune response already in progress or may involve preventing the induction of an immune response. Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions), e.g., modulating or preventing high level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The administration of a therapeutic composition of the invention may prevent cytokine synthesis by immune cells, such as T cells, and thus acts as an immunosuppressant. Moreover, a lack of costimulation may also be sufficient to anergize the T cells, thereby inducing tolerance in a subject. Induction of long-term tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or tolerance in a subject, it may also be necessary to block the function of a combination of B lymphocyte antigens.

The efficacy of particular therapeutic compositions in preventing organ transplant rejection or GVHD can be assessed using animal models that are predictive of efficacy in humans. Examples of appropriate systems which can be used include allogeneic cardiac grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive effects of CTLA4Ig fusion proteins in vivo as described in Lenschow et al., Science 257:789-792 (1992) and Turka et al., Proc. Natl.

Acad. Sci USA, 89:11102-11105 (1992), herein incorporated by reference in their entirety. In addition, murine models of GVHD (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 846-847, herein incorporated by reference in its entirety) can be used to determine the effect of therapeutic compositions of the invention on the development of that disease.

4.8.10.5 ADMINISTRATION

The anti- JPL monoclonal antibodies used in the practice of a method of the invention may be formulated into pharmaceutical compositions comprising a carrier suitable for the desired delivery method. Suitable carriers include any material which when combined with the anti-JPL antibodies retains the anti-tumor function of the antibody and is nonreactive with the subject's immune systems. Examples include, but are not limited to, any of a number of standard pharmaceutical carriers such as sterile phosphate buffered saline solutions, bacteriostatic water, and the like.

The anti-JPL antibody formulations may be administered via any route capable of delivering the antibodies to the tumor site. Potentially effective routes of administration include, but are not limited to, intravenous, intraperitoneal, intramuscular, intratumor, intradermal, and the like. The preferred route of administration is by intravenous injection. A preferred formulation for intravenous injection comprises anti- JPL mAbs in a solution of preserved bacteriostatic water, sterile unpreserved water, and/or diluted in polyvinylchloride or polyethylene bags containing 0.9% sterile sodium chloride for Injection, USP. The anti-JPL mAb preparation may be lyophilized and stored as a sterile powder, preferably under vacuum, and then reconstituted in bacteriostatic water containing, for example, benzyl alcohol preservative, or in sterile water prior to injection.

Treatment will generally involve the repeated administration of the anti-JPL antibody preparation via an acceptable route of administration such as intravenous injection (IV), typically at a dose in the range of about 0.1 to about 10 mg/kg body weight; however other exemplary doses in the range of 0.01 mg/kg to about 100 mg/kg are also contemplated. Doses in the range of 10-500 mg mAb per week may be effective and well tolerated. Rituximab (Rituxan®), a chimeric CD20 antibody used to treat B-cell lymphoma, non-Hodgkin's lymphoma, and relapsed indolent lymphoma, is typically

administered at 375 mg/m^2 by IV infusion once a week for 4 to 8 doses. Sometimes a second course is necessary, but no more than 2 courses are allowed. An effective dosage range for Rituxan® would be 50 to 500 mg/m^2 (Maloney, *et al., Blood* 84: 2457-2466 (1994); Davis, *et al., J. Clin. Oncol.* 18: 3135-3143 (2000), herein incorporated by reference in their entirety). Based on clinical experience with Trastuzumab (Herceptin®), a humanized monoclonal antibody used to treat HER2 (human epidermal growth factor 2)-positive metastatic breast cancer (Slamon, *et al., Mol Cell Biol.* 9: 1165 (1989), herein incorporated by reference in its entirety), an initial loading dose of approximately 4 mg/kg patient body weight IV followed by weekly doses of about 2 mg/kg IV of the anti- JPL polypeptide mAb preparation may represent an acceptable dosing regimen (Slamon, *et al., N. Engl. J. Med.* 344: 783(2001), herein incorporated by reference in its entirety). Preferably, the initial loading dose is administered as a 90 minute or longer infusion. The periodic maintenance dose may be administered as a 30 minute or longer infusion, provided the initial dose was well tolerated. However, as one of skill in the art will understand, various factors will influence the ideal dose regimen in a particular case. Such factors may include, for example, the binding affinity and half life of the mAb or mAbs used, the degree of JPL polypeptide overexpression in the patient, the extent of circulating shed JPL antigen, the desired steady-state antibody concentration level, frequency of treatment, and the influence of chemotherapeutic agents used in combination with the treatment method of the invention.

Treatment can also involve anti- JPL antibodies conjugated to radioisotopes. Studies using radiolabeled-anticarcinoembryonic antigen (anti-CEA) monoclonal antibodies, provide a dosage guideline for tumor regression of 2-3 infusions of 30-80 mCi/m^2 (Behr, *et al. Clin, Cancer Res.* 5(10 Suppl.): 3232s-3242s (1999), Juweid, *et al., J. Nucl. Med.* 39:34-42 (1998), herein incorporated by reference in their entirety).

Alternatively, dendritic cells transfected with mRNA encoding a JPL polypeptide can be used as a vaccine to stimulate T-cell mediated anti-tumor responses. Studies with dendritic cells transfected with prostate-specific antigen mRNA suggest a 3 cycles of intravenous administration of $1\times10^7 - 5\times10^7$ cells for 2-6 weeks concomitant with an intradermal injection of 10^7 cells may provide a suitable dosage regimen (Heiser, *et al., J. Clin. Invest.* 109:409-417 (2002); Hadzantonis and O'Neill, *Cancer Biother.*

Radiopharm. 1:11-22 (1999), herein incorporated by reference in their entirety). Other exemplary doses of between 1×10^{5} to 1×10^{9} or 1×10^{6} to 1×10^{8} cells are also contemplated.

Naked DNA vaccines using plasmids encoding JPL polypeptides can induce an immunologic anti-tumor response. Administration of naked DNA by direct injection into the skin and muscle is not associated with limitations encountered using viral vectors, such as the development of adverse immune reactions and risk of insertional mutagenesis (Hengge, *et al., J. Invest. Dermatol.* 116:979 (2001), herein incorporated by reference in its entirety). Studies have shown that direct injection of exogenous cDNA into muscle tissue results in a strong immune response and protective immunity (Ilan, *Curr. Opin. Mol. Ther.* 1:116-120 (1999), herein incorporated by reference in its entirety). Physical (gene gun, electroporation) and chemical (cationic lipid or polymer) approaches have been developed to enhance efficiency and target cell specificity of gene transfer by plasmid DNA (Nishikawa and Huang, *Hum. Gene Ther.* 12:861-870 (2001), herein incorporated by reference in its entirety). Plasmid DNA can also be administered to the lungs by aerosol delivery (Densmore, *et al., Mol. Ther.* 1:180-188 (2000), herein incorporated by reference in its entirety). Gene therapy by direct injection of naked or lipid – coated plasmid DNA is envisioned for the prevention, treatment, and cure of diseases such as cancer, acquired immunodeficiency syndrome, cystic fibrosis, cerebrovascular disease, and hypertension (Prazeres, *et al., Trends Biotechnol.* 17:169-174 (1999); Weihl, *et al., Neurosurgery* 44:239-252 (1999), herein incorporated by reference in their entirety). HIV-1 DNA vaccine dose-escalating studies indicate administration of 30-300 μg/dose as a suitable therapy (Weber, *et al.*, Eur. J. Clin. Microbiol. Infect. Dis. 20: 800 (2001), herein incorporated by reference in its entirety. Naked DNA injected intracerebrally into the mouse brain was shown to provide expression of a reporter protein, wherein expression was dose-dependent and maximal for 150 μg DNA injected (Schwartz, *et al., Gene Ther.* 3:405-411 (1996), herein incorporated by reference in its entirety) Gene expression in mice after intramuscular injection of nanospheres containing 1 microgram of beta-galactosidase plasmid was greater and more prolonged than was observed after an injection with an equal amount of naked DNA or DNA complexed with Lipofectamine (Truong, *et al., Hum. Gene Ther.*

9:1709-1717 (1998), herein incorporated by reference in its entirety). In a study of plasmid-mediated gene transfer into skeletal muscle as a means of providing a therapeutic source of insulin, wherein four plasmid constructs comprising a mouse furin cDNA transgene and rat proinsulin cDNA were injected into the calf muscles of male Balb/c mice, the optimal dose for most constructs was 100 micrograms plasmid DNA (Kon, *et al. J. Gene Med.* 1:186-194 (1999), herein incorporated by reference in its entirety). Other exemplary doses of 1-1000 μg/dose or 10-500 μg/dose are also contemplated.

Optimally, patients should be evaluated for the level of circulating shed JPL antigen in serum in order to assist in the determination of the most effective dosing regimen and related factors. Such evaluations may also be used for monitoring purposes throughout therapy, and may be useful to gauge therapeutic success in combination with evaluating other parameters.

A. TARGETING COMPOSITIONS

Compositions for targeting JPL-expressing cells are within the scope of the present invention. Pharmaceutical compositions comprising antibodies are described in detail in, for example, US Patent No. 6,171,586 to Lam *et al.*, issued January 9, 2001 (herein incorporated by reference in its entirety). Such compositions comprise a therapeutically or prophylactically effective amount an antibody, or a fragment, variant, derivative or fusion thereof as described herein, in admixture with a pharmaceutically acceptable agent. Typically, the JPL targeting agent will be sufficiently purified for administration to an animal.

The pharmaceutical composition may contain formulation materials for modifying, maintaining or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption or penetration of the composition. Suitable formulation materials include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine or lysine); antimicrobials; antioxidants (such as ascorbic acid, sodium sulfite or sodium hydrogen-sulfite); buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates, other organic acids); bulking agents (such as mannitol or glycine), chelating agents [such as ethylenediamine tetraacetic acid (EDTA)]; complexing agents (such as caffeine,

polyvinylpyrrolidone, beta-cyclodextrin or hydroxypropyl-beta-cyclodextrin); fillers; monosaccharides; disaccharides and other carbohydrates (such as glucose, mannose, or dextrins); proteins (such as serum albumin, gelatin or immunoglobulins); coloring; flavoring and diluting agents; emulsifying agents; hydrophilic polymers (such as polyvinylpyrrolidone); low molecular weight polypeptides; salt-forming counterions (such as sodium); preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide); solvents (such as glycerin, propylene glycol or polyethylene glycol); sugar alcohols (such as mannitol or sorbitol); suspending agents; surfactants or wetting agents (such as pluronics, PEG, sorbitan esters, polysorbates such as polysorbate 20, polysorbate 80, triton, tromethamine, lecithin, cholesterol, tyloxapal); stability enhancing agents (sucrose or sorbitol); tonicity enhancing agents (such as alkali metal halides (preferably sodium or potassium chloride, mannitol sorbitol); delivery vehicles; diluents; excipients and/or pharmaceutical adjuvants. (*Remington's Pharmaceutical Sciences,* 18th Edition, Ed. A.R. Gennaro, Mack Publishing Company, (1990)).

The optimal pharmaceutical composition will be determined by one skilled in the art depending upon, for example, the intended route of administration, delivery format, and desired dosage. *See*, for example, *Remington's Pharmaceutical Sciences*, *supra.* Such compositions may influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of the JPL targeting agent.

The primary vehicle or carrier in a pharmaceutical composition may be either aqueous or non-aqueous in nature. For example, a suitable vehicle or carrier may be water for injection, physiological saline solution or artificial cerebrospinal fluid, possibly supplemented with other materials common in compositions for parenteral administration. Neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles. Other exemplary pharmaceutical compositions comprise Tris buffer of about pH 7.0-8.5, or acetate buffer of about pH 4.0-5.5, which may further include sorbitol or a suitable substitute therefor. In one embodiment of the present invention, JPL targeting agent compositions may be prepared for storage by mixing the selected composition having the desired degree of purity with optional formulation agents (*Remington's Pharmaceutical Sciences*, *supra*) in the form of a lyophilized cake or an

aqueous solution. Further, the binding agent product may be formulated as a lyophilizate using appropriate excipients such as sucrose.

The pharmaceutical compositions can be selected for parenteral delivery. Alternatively, the compositions may be selected for inhalation or for delivery through the digestive tract, such as orally. The preparation of such pharmaceutically acceptable compositions is within the skill of the art. The formulation components are present in concentrations that are acceptable to the site of administration. For example, buffers are used to maintain the composition at physiological pH or at slightly lower pH, typically within a pH range of from about 5 to about 8. When parenteral administration is contemplated, the therapeutic compositions for use in this invention may be in the form of a pyrogen-free, parenterally acceptable aqueous solution comprising the JPL targeting agent in a pharmaceutically acceptable vehicle. A particularly suitable vehicle for parenteral injection is sterile distilled water in which a JPL targeting agent is formulated as a sterile, isotonic solution, properly preserved. Yet another preparation can involve the formulation of the desired molecule with an agent, such as injectable microspheres, bio-erodible particles, polymeric compounds (polylactic acid, polyglycolic acid), beads, or liposomes, that provides for the controlled or sustained release of the product which may then be delivered via a depot injection. Hyaluronic acid may also be used, and this may have the effect of promoting sustained duration in the circulation. Other suitable means for the introduction of the desired molecule include implantable drug delivery devices.

In another aspect, pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils, such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate, triglycerides, or liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also contain suitable stabilizers or agents to increase the solubility of the compounds and allow for the preparation of highly concentrated solutions.

In another embodiment, a pharmaceutical composition may be formulated for inhalation. For example, a JPL targeting agent may be formulated as a dry powder for inhalation. Polypeptide or nucleic acid molecule inhalation solutions may also be formulated with a propellant for aerosol delivery. In yet another embodiment, solutions may be nebulized. Pulmonary administration is further described in PCT Application No. PCT/US94/001875, (herein incorporated by reference in its entirety) which describes pulmonary delivery of chemically modified proteins.

It is also contemplated that certain formulations may be administered orally. In one embodiment of the present invention, JPL targeting agents that are administered in this fashion can be formulated with or without those carriers customarily used in the compounding of solid dosage forms such as tablets and capsules. For example, a capsule may be designed to release the active portion of the formulation at the point in the gastrointestinal tract when bioavailability is maximized and pre-systemic degradation is minimized. Additional agents can be included to facilitate absorption of the binding agent molecule. Diluents, flavorings, low melting point waxes, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and binders may also be employed.

Pharmaceutical compositions for oral administration can also be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combining active compounds with solid excipient and processing the resultant mixture of granules (optionally, after grinding) to obtain tablets or dragee cores. Suitable auxiliaries can be added, if desired. Suitable excipients include carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, and sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums, including arabic and tragacanth; and proteins, such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, and alginic acid or a salt thereof, such as sodium alginate.

Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations that can be used orally also include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with fillers or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the JPL targeting agent may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Another pharmaceutical composition may involve an effective quantity of JPL targeting agent in a mixture with non-toxic excipients that are suitable for the manufacture of tablets. By dissolving the tablets in sterile water, or other appropriate vehicle, solutions can be prepared in unit dose form. Suitable excipients include, but are not limited to, inert diluents, such as calcium carbonate, sodium carbonate or bicarbonate, lactose, or calcium phosphate; or binding agents, such as starch, gelatin, or acacia; or lubricating agents such as magnesium stearate, stearic acid, or talc.

Additional pharmaceutical compositions will be evident to those skilled in the art, including formulations involving JPL targeting agents in sustained- or controlled-delivery formulations. Techniques for formulating a variety of other sustained- or controlled-delivery means, such as liposome carriers, bio-erodible microparticles or porous beads and depot injections, are also known to those skilled in the art. *See*, for example, PCT/US93/00829 (herein incorporated by reference in its entirety) that describes controlled release of porous polymeric microparticles for the delivery of pharmaceutical compositions. Additional examples of sustained-release preparations include semipermeable polymer matrices in the form of shaped articles, e.g. films, or microcapsules. Sustained release matrices may include polyesters, hydrogels, polylactides (U.S. Patent No. 3,773,919; European Patent EP 58,481), copolymers of L-

glutamic acid and gamma ethyl-L-glutamate (Sidman *et al., Biopolymers*, 22:547-556 (1983)), poly (2-hydroxyethyl-methacrylate) (Langer *et al., J Biomed Mater Res*, 15:167-277, (1981)) and (Langer *et al., Chem Tech*, 12:98-105(1982)), ethylene vinyl acetate (Langer *et al., supra*) or poly-D (-)-3-hydroxybutyric acid (European Patent EP 133,988), all of which are herein incorporated by reference in their entirety. Sustained-release compositions also include liposomes, which can be prepared by any of several methods known in the art. See e.g., Epstein, *et al., Proc Natl Acad Sci (USA)*, 82:3688-3692 (1985); European Patents EP 36,676; EP 88,046; EP 143,949, all of which are herein incorporated by reference in their entirety.

The pharmaceutical composition to be used for *in vivo* administration typically must be sterile. This may be accomplished by filtration through sterile filtration membranes. Where the composition is lyophilized, sterilization using this method may be conducted either prior to or following lyophilization and reconstitution. The composition for parenteral administration may be stored in lyophilized form or in solution. In addition, parenteral compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Once the pharmaceutical composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or a dehydrated or lyophilized powder. Such formulations may be stored either in a ready-to-use form or in a form (e.g., lyophilized) requiring reconstitution prior to administration.

In a specific embodiment, the present invention is directed to kits for producing a single-dose administration unit. The kits may each contain both a first container having a dried JPL targeting agent and a second container having an aqueous formulation. Also included within the scope of this invention are kits containing single and multi-chambered pre-filled syringes (e.g., liquid syringes and lyosyringes).

B. DOSAGE

An effective amount of a pharmaceutical composition to be employed therapeutically will depend, for example, upon the therapeutic context and objectives. One skilled in the art will appreciate that the appropriate dosage levels for treatment will

thus vary depending, in part, upon the molecule delivered, the indication for which the JPL targeting agent is being used, the route of administration, and the size (body weight, body surface or organ size) and condition (the age and general health) of the patient. Accordingly, the clinician may titer the dosage and modify the route of administration to obtain the optimal therapeutic effect. A typical dosage may range from about 0.1 mg/kg to up to about 100 mg/kg or more, depending on the factors mentioned above. In other embodiments, the dosage may range from 0.1 mg/kg up to about 100 mg/kg; or 0.01 mg/kg to 1 g/kg; or 1 mg/kg up to about 100 mg/kg or 5 mg/kg up to about 100 mg/kg. In other embodiments, the dosage may range from 10 mCi to 100 mCi per dose for radioimmunotherapy, from about $1\times10^7 - 5\times10^7$ cells or 1×10^5 to 1×10^9 cells or 1×10^6 to 1×10^8 cells per injection or infusion, or from 30 μg to 300 μg naked DNA per dose or 1-1000 μg/dose or 10-500 μg/dose, depending on the factors listed above.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models such as mice, rats, rabbits, dogs, or pigs. An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

The exact dosage will be determined in light of factors related to the subject requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the active compound or to maintain the desired effect. Factors that may be taken into account include the severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or biweekly depending on the half-life and clearance rate of the particular formulation.

The frequency of dosing will depend upon the pharmacokinetic parameters of the JPL targeting agent in the formulation used. Typically, a composition is administered until a dosage is reached that achieves the desired effect. The composition may therefore be administered as a single dose, or as multiple doses (at the same or different concentrations/dosages) over time, or as a continuous infusion. Further refinement of the

appropriate dosage is routinely made. Appropriate dosages may be ascertained through use of appropriate dose-response data.

C. ROUTES OF ADMINISTRATION

The route of administration of the pharmaceutical composition is in accord with known methods, e.g. orally, through injection by intravenous, intraperitoneal, intracerebral (intra-parenchymal), intracerebroventricular, intramuscular, intra-ocular, intra-arterial, intraportal, intralesional routes, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, urethral, vaginal, or rectal means, by sustained release systems or by implantation devices. Where desired, the compositions may be administered by bolus injection or continuously by infusion, or by implantation device.

Alternatively or additionally, the composition may be administered locally via implantation of a membrane, sponge, or another appropriate material on to which the JPL targeting agent has been absorbed or encapsulated. Where an implantation device is used, the device may be implanted into any suitable tissue or organ, and delivery of the JPL targeting agent may be via diffusion, timed-release bolus, or continuous administration.

In some cases, it may be desirable to use pharmaceutical compositions in an *ex vivo* manner. In such instances, cells, tissues, or organs that have been removed from the patient are exposed to the pharmaceutical compositions after which the cells, tissues and/or organs are subsequently implanted back into the patient.

In other cases, a JPL targeting agent can be delivered by implanting certain cells that have been genetically engineered to express and secrete the polypeptide. Such cells may be animal or human cells, and may be autologous, heterologous, or xenogeneic. Optionally, the cells may be immortalized. In order to decrease the chance of an immunological response, the cells may be encapsulated to avoid infiltration of surrounding tissues. The encapsulation materials are typically biocompatible, semi-permeable polymeric enclosures or membranes that allow the release of the protein product(s) but prevent the destruction of the cells by the patient's immune system or by other detrimental factors from the surrounding tissues.

4.8.10.6 COMBINATION THERAPY

JPL targeting agents of the invention can be utilized in combination with other therapeutic agents, and may enhance the effect of these other therapeutic agents such that a lesser daily amount, lesser total amount or reduced frequency of administration is required in order to achieve the same therapeutic effect at reduced toxicity. These other therapeutics include, for example radiation treatment, chemotherapeutic agents, as well as other growth factors.

In one embodiment, anti- JPL antibody is used as a radiosensitizer. In such embodiments, the anti- JPL antibody is conjugated to a radiosensitizing agent. The term "radiosensitizer," as used herein, is defined as a molecule, preferably a low molecular weight molecule, administered to animals in therapeutically effective amounts to increase the sensitivity of the cells to be radiosensitized to electromagnetic radiation and/or to promote the treatment of diseases that are treatable with electromagnetic radiation. Diseases that are treatable with electromagnetic radiation include neoplastic diseases, benign and malignant tumors, and cancerous cells.

The terms "electromagnetic radiation" and "radiation" as used herein include, but are not limited to, radiation having the wavelength of 10^{-20} to 100 meters. Preferred embodiments of the present invention employ the electromagnetic radiation of: gamma-radiation (10^{-20} to 10^{-13} m), X-ray radiation (10^{-12} to 10^{-9} m), ultraviolet light (10 nm to 400 nm), visible light (400 nm to 700 nm), infrared radiation (700 nm to 1.0 mm), and microwave radiation (1 mm to 30 cm).

Radiosensitizers are known to increase the sensitivity of cancerous cells to the toxic effects of electromagnetic radiation. Many cancer treatment protocols currently employ radiosensitizers activated by the electromagnetic radiation of X-rays. Examples of X-ray activated radiosensitizers include, but are not limited to, the following: metronidazole, misonidazole, desmethylmisonidazole, pimonidazole, etanidazole, nimorazole, mitomycin C, RSU 1069, SR 4233, EO9, RB 6145, nicotinamide, 5-bromodeoxyuridine (BUdR), 5-iododeoxyuridine (IUdR), bromodeoxycytidine, fluorodeoxyuridine (FUdR), hydroxyurea, cisplatin, and therapeutically effective analogs and derivatives of the same.

Photodynamic therapy (PDT) of cancers employs visible light as the radiation activator of the sensitizing agent. Examples of photodynamic radiosensitizers include the following, but are not limited to: hematoporphyrin derivatives, Photofrin(r), benzoporphyrin derivatives, NPe6, tin etioporphyrin (SnET2), pheoborbide-a, bacteriochlorophyll-a, naphthalocyanines, phthalocyanines, zinc phthalocyanine, and therapeutically effective analogs and derivatives of the same.

Chemotherapy treatment can employ anti-neoplastic agents including, for example, alkylating agents including: nitrogen mustards, such as mechlorethamine, cyclophosphamide, ifosfamide, melphalan and chlorambucil; nitrosoureas, such as carmustine (BCNU), lomustine (CCNU), and semustine (methyl-CCNU); ethylenimines/methylmelamine such as thriethylenemelamine (TEM), triethylene, thiophosphoramide (thiotepa), hexamethylmelamine (HMM, altretamine); alkyl sulfonates such as busulfan; triazines such as dacarbazine (DTIC); antimetabolites including folic acid analogs such as methotrexate and trimetrexate, pyrimidine analogs such as 5-fluorouracil, fluorodeoxyuridine, gemcitabine, cytosine arabinoside (AraC, cytarabine), 5-azacytidine, 2,2´-difluorodeoxycytidine, purine analogs such as 6-mercaptopurine, 6-thioguanine, azathioprine, 2'-deoxycoformycin (pentostatin), erythrohydroxynonyladenine (EHNA), fludarabine phosphate, and 2-chlorodeoxyadenosine (cladribine, 2-CdA); natural products including antimitotic drugs such as paclitaxel, vinca alkaloids including vinblastine (VLB), vincristine, and vinorelbine, taxotere, estramustine, and estramustine phosphate; ppipodophylotoxins such as etoposide and teniposide; antibiotics such as actimomycin D, daunomycin (rubidomycin), doxorubicin, mitoxantrone, idarubicin, bleomycins, plicamycin (mithramycin), mitomycinC, and actinomycin; enzymes such as L-asparaginase; biological response modifiers such as interferon-alpha, IL-2, G-CSF and GM-CSF; miscellaneous agents including platinium coordination complexes such as cisplatin and carboplatin, anthracenediones such as mitoxantrone, substituted urea such as hydroxyurea, methylhydrazine derivatives including N-methylhydrazine (MIH) and procarbazine, adrenocortical suppressants such as mitotane (o,p´-DDD) and aminoglutethimide; hormones and antagonists including adrenocorticosteroid antagonists such as prednisone and equivalents, dexamethasone and aminoglutethimide; progestin

such as hydroxyprogesterone caproate, medroxyprogesterone acetate and megestrol acetate; estrogen such as diethylstilbestrol and ethinyl estradiol equivalents; antiestrogen such as tamoxifen; androgens including testosterone propionate and fluoxymesterone/equivalents; antiandrogens such as flutamide, gonadotropin-releasing hormone analogs and leuprolide; and non-steroidal antiandrogens such as flutamide.

Combination therapy with growth factors can include cytokines, lymphokines, growth factors, or other hematopoietic factors such as M-CSF, GM-CSF, TNF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IFN, TNF0, TNF1, TNF2, G-CSF, Meg-CSF, GM-CSF, thrombopoietin, stem cell factor, and erythropoietin. Other compositions can include known angiopoietins, for example, vascular endothelial growth factor (VEGF). Growth factors include angiogenin, bone morphogenic protein-1, bone morphogenic protein-2, bone morphogenic protein-3, bone morphogenic protein-4, bone morphogenic protein-5, bone morphogenic protein-6, bone morphogenic protein-7, bone morphogenic protein-8, bone morphogenic protein-9, bone morphogenic protein-10, bone morphogenic protein-11, bone morphogenic protein-12, bone morphogenic protein-13, bone morphogenic protein-14, bone morphogenic protein-15, bone morphogenic protein receptor IA, bone morphogenic protein receptor IB, brain derived neurotrophic factor, ciliary neutrophic factor, ciliary neutrophic factor receptor , cytokine-induced neutrophil chemotactic factor 1, cytokine-induced neutrophil chemotactic factor 2, endothelial cell growth factor, endothelin 1, epidermal growth factor, epithelial-derived neutrophil attractant, fibroblast growth factor 4, fibroblast growth factor 5, fibroblast growth factor 6, fibroblast growth factor 7, fibroblast growth factor 8, fibroblast growth factor 8b, fibroblast growth factor 8c, fibroblast growth factor 9, fibroblast growth factor 10, fibroblast growth factor acidic, fibroblast growth factor basic, glial cell line-derived neutrophic factor receptor 1, glial cell line-derived neutrophic factor receptor 2, growth related protein, heparin binding epidermal growth factor, hepatocyte growth factor, hepatocyte growth factor receptor, insulin-like growth factor I, insulin-like growth factor receptor, insulin-like growth factor II, insulin-like growth factor binding protein, keratinocyte growth factor, leukemia inhibitory factor, leukemia inhibitory factor receptor, nerve growth factor nerve growth factor receptor, neurotrophin-3, neurotrophin-4, placenta growth factor, placenta growth

factor 2, platelet-derived endothelial cell growth factor, platelet derived growth factor, platelet derived growth factor A chain, platelet derived growth factor AA, platelet derived growth factor AB, platelet derived growth factor B chain, platelet derived growth factor BB, platelet derived growth factor receptor, pre-B cell growth stimulating factor, stem cell factor, stem cell factor receptor, transforming growth factor, transforming growth factor 1, transforming growth factor 1.2, transforming growth factor 2, transforming growth factor 3, transforming growth factor 5, latent transforming growth factor 1, transforming growth factor binding protein I, transforming growth factor binding protein II, transforming growth factor binding protein III, tumor necrosis factor receptor type I, tumor necrosis factor receptor type II, urokinase-type plasminogen activator receptor, vascular endothelial growth factor, and chimeric proteins and biologically or immunologically active fragments thereof.

4.8.10.7 DIAGNOSTIC USES OF JPL POLYPEPTIDES

A. ASSAYS FOR DETERMINING JPL EXPRESSION STATUS

Determining the status of JPL expression patterns in an individual may be used to diagnose melanoma and may provide prognostic information useful in defining appropriate therapeutic options. Similarly, the expression status of JPL may provide information useful for predicting susceptibility to particular disease stages, progression, and/or tumor aggressiveness. The invention provides methods and assays for determining JPL expression status and diagnosing melanomas and other cancers that express JPL polypeptides and polynucleotides.

In one aspect, the invention provides assays useful in determining the presence of cancer in an individual, comprising detecting a significant increase in JPL mRNA or protein expression in a test cell or tissue or fluid sample relative to expression levels in the corresponding normal cell or tissue. In one embodiment, the presence of JPL mRNA is evaluated in tissue samples of a melanoma. The presence of significant JPL mRNA expression may be useful to indicate whether the melanoma is susceptible to JPL polypeptide immunotargeting. In a related embodiment, JPL polypeptide expression status may be determined at the protein level rather than at the nucleic acid level. For example, such a method or assay would comprise determining the level of JPL

polypeptide expressed by cells in a test tissue sample and comparing the level so determined to the level of JPL polypeptide expressed in a corresponding normal sample. In one embodiment, the presence of JPL polypeptide is evaluated, for example, using immunohistochemical methods. JPL polypeptide antibodies capable of detecting JPL expression may be used in a variety of assay formats well known in the art for this purpose.

Peripheral blood may be conveniently assayed for the presence of melanoma cancer cells using RT-PCR to detect JPL mRNA expression. The presence of RT-PCR amplifiable JPL mRNA provides an indication of the presence of one of these types of cancer. A sensitive assay for detecting and characterizing carcinoma cells in blood may be used (Racila, *et al., Proc. Natl. Acad. Sci. USA* 95: 4589-4594 (1998)). This assay combines immunomagnetic enrichment with multiparameter flow cytometric and immunohistochemical analyses, and is highly sensitive for the detection of cancer cells in blood, reportedly capable of detecting one epithelial cell in 1 ml of peripheral blood.

A related aspect of the invention is directed to predicting susceptibility to developing melanoma in an individual. In one embodiment, a method for predicting susceptibility to cancer comprises detecting JPL mRNA or JPL polypeptide in a tissue sample, its presence indicating susceptibility to cancer, wherein the degree of JPL mRNA expression present is proportional to the degree of susceptibility.

Yet another related aspect of the invention is directed to methods for assessment of tumor aggressiveness (Orlandi, *et al.*, *Cancer Res.* 62:567 (2002), herin incorporated by reference in its entirety). In one embodiment, a method for gauging aggressiveness of a melanoma tumor comprises determining the level of JPL mRNA or JPL protein expressed by cells in a sample of the tumor, comparing the level so determined to the level of JPL mRNA or JPL protein expressed in a corresponding normal tissue taken from the same individual or a normal tissue reference sample, wherein the degree of JPL mRNA or JPL protein expression in the tumor sample relative to the normal sample indicates the degree of aggressiveness.

Methods for detecting and quantifying the expression of JPL mRNA or protein are described herein and use standard nucleic acid and protein detection and quantification technologies well known in the art. Standard methods for the detection

and quantification of JPL polypeptides mRNA include *in situ* hybridization using labeled JPL riboprobes (Gemou-Engesaeth, *et al., Pediatrics*, 109:E24-E32 (2002)), Northern blot and related techniques using JPL polynucleotide probes (Kunzli, *et al., Cancer* 94:228 (2002)) , RT-PCR analysis using primers specific for JPL polypeptides (Angchaiskisiri, *et al., Blood* 99:130 (2002)), and other amplification type detection methods, such as, for example, branched DNA (Jardi, *et al., J. Viral Hepat.* 8:465-471 (2001)), SISBA, TMA (Kimura, *et al., J. Clin. Microbiol.* 40:439-445 (2002)), and microarray products of a variety of sorts, such as oligos, cDNAs, and monoclonal antibodies. In a specific embodiment, real-time RT-PCR may be used to detect and quantify JPL polypeptide mRNA expression (Simpson, *et al., Molec. Vision* 6:178-183 (2000)). Standard methods for the detection and quantification of protein may be used for this purpose. In a specific embodiment, polyclonal or monoclonal antibodies specifically reactive with the wild-type JPL polypeptide may be used in an immunohistochemical assay of biopsied tissue (Ristimaki, *et al., Cancer Res.* 62:632 (2002), herein incorporated by reference in its entirety).

B. MEDICAL IMAGING

JPL antibodies that recognize JPL and fragments thereof are useful in medical imaging of sites expressing JPL polypeptides. Such methods involve chemical attachment of a labeling or imaging agent, such as a radioisotope, which include ^{67}Cu, ^{90}Y, ^{125}I, ^{131}I, ^{186}Re, ^{188}Re, ^{211}At, ^{212}Bi, administration of the labeled antibody and fragment to a subject in a pharmaceutically acceptable carrier, and imaging the labeled antibody and fragment *in vivo* at the target site. Radiolabelled anti- JPL antibodies or fragments thereof may be particularly useful in *in vivo* imaging of JPL expressing cancers, such as melanomas. Such antibodies may provide highly sensitive methods for detecting metastasis of JPL-expressing cancers.

Upon consideration of the present disclosure, one of skill in the art will appreciate that many other embodiments and variations may be made in the scope of the present invention. Accordingly, it is intended that the broader aspects of the present invention not be limited to the disclosure of the following examples.

4.8.11 RECEPTOR/LIGAND ACTIVITY

A polypeptide of the present invention may also demonstrate activity as receptor, receptor ligand or inhibitor or agonist of receptor/ligand interactions. A polynucleotide of the invention can encode a polypeptide exhibiting such characteristics. Examples of such receptors and ligands include, without limitation, cytokine receptors and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, receptors involved in cell-cell interactions and their ligands (including without limitation, cellular adhesion molecules (such as selectins, integrins and their ligands) and receptor/ligand pairs involved in antigen presentation, antigen recognition and development of cellular and humoral immune responses. Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction. A protein of the present invention (including, without limitation, fragments of receptors and ligands) may themselves be useful as inhibitors of receptor/ligand interactions.

The activity of a polypeptide of the invention may, among other means, be measured by the following methods:

Suitable assays for receptor-ligand activity include without limitation those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley- Interscience (Chapter 7.28, Measurement of Cellular Adhesion under static conditions 7.28.1- 7.28.22), Takai et al., Proc. Natl. Acad. Sci. USA 84:6864-6868, 1987; Bierer et al., J. Exp. Med. 168:1145-1156, 1988; Rosenstein et al., J. Exp. Med. 169:149-160 1989; Stoltenborg et al., J. Immunol. Methods 175:59-68, 1994; Stitt et al., Cell 80:661-670, 1995.

By way of example, the polypeptides of the invention may be used as a receptor for a ligand(s) thereby transmitting the biological activity of that ligand(s). Ligands may be identified through binding assa ys, affinity chromatography, dihybrid screening assays, BIAcore assays, gel overlay assays, or other methods known in the art.

Studies characterizing drugs or proteins as agonist or antagonist or partial agonists or a partial antagonist require the use of other proteins as competing ligands. The polypeptides of the present invention or ligand(s) thereof may be labeled by being

coupled to radioisotopes, colorimetric molecules or a toxin molecules by conventional methods. ("Guide to Protein Purification" Murray P. Deutscher (ed) Methods in Enzymology Vol. 182 (1990) Academic Press, Inc. San Diego). Examples of radioisotopes include, but are not limited to, tritium and carbon-14 . Examples of colorimetric molecules include, but are not limited to, fluorescent molecules such as fluorescamine, or rhodamine or other colorimetric molecules. Examples of toxins include, but are not limited, to ricin.

4.8.12 DRUG SCREENING

This invention is particularly useful for screening chemical compounds by using the novel polypeptides or binding fragments thereof in any of a variety of drug screening techniques. The polypeptides or fragments employed in such a test may either be free in solution, affixed to a solid support, borne on a cell surface or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the polypeptide or a fragment thereof. Drugs are screened against such transformed cells in competitive binding assays. Such cells, either in viable or fixed form, can be used for standard binding assays. One may measure, for example, the formation of complexes between polypeptides of the invention or fragments and the agent being tested or examine the diminution in complex formation between the novel polypeptides and an appropriate cell line, which are well known in the art.

Sources for test compounds that may be screened for ability to bind to or modulate (i.e., increase or decrease) the activity of polypeptides of the invention include (1) inorganic and organic chemical libraries, (2) natural product libraries, and (3) combinatorial libraries comprised of either random or mimetic peptides, oligonucleotides or organic molecules.

Chemical libraries may be readily synthesized or purchased from a number of commercial sources, and may include structural analogs of known compounds or compounds that are identified as "hits" or "leads" via natural product screening.

The sources of natural product libraries are microorganisms (including bacteria and fungi), animals, plants or other vegetation, or marine organisms, and libraries of

mixtures for screening may be created by: (1) fermentation and extraction of broths from soil, plant or marine microorganisms or (2) extraction of the organisms themselves. Natural product libraries include polyketides, non-ribosomal peptides, and (non-naturally occurring) variants thereof. For a review, see *Science 282*:63-68 (1998).

Combinatorial libraries are composed of large numbers of peptides, oligonucleotides or organic compounds and can be readily prepared by traditional automated synthesis methods, PCR, cloning or proprietary synthetic methods. Of particular interest are peptide and oligonucleotide combinatorial libraries. Still other libraries of interest include peptide, protein, peptidomimetic, multiparallel synthetic collection, recombinatorial, and polypeptide libraries. For a review of combinatorial chemistry and libraries created therefrom, see Myers, *Curr. Opin. Biotechnol.* 8:701-707 (1997). For reviews and examples of peptidomimetic libraries, see Al-Obeidi et al., *Mol. Biotechnol*, 9(3):205-23 (1998); Hruby et al., *Curr Opin Chem Biol*, 1(1):114-19 (1997); Dorner et al., *Bioorg Med Chem*, 4(5):709-15 (1996) (alkylated dipeptides).

Identification of modulators through use of the various libraries described herein permits modification of the candidate "hit" (or "lead") to optimize the capacity of the "hit" to bind a polypeptide of the invention. The molecules identified in the binding assay are then tested for antagonist or agonist activity in *in vivo* tissue culture or animal models that are well known in the art. In brief, the molecules are titrated into a plurality of cell cultures or animals and then tested for either cell/animal death or prolonged survival of the animal/cells.

The binding molecules thus identified may be complexed with toxins, e.g., ricin or cholera, or with other compounds that are toxic to cells such as radioisotopes. The toxin-binding molecule complex is then targeted to a tumor or other cell by the specificity of the binding molecule for a polypeptide of the invention. Alternatively, the binding molecules may be complexed with imaging agents for targeting and imaging purposes.

4.8.13 ASSAY FOR RECEPTOR ACTIVITY

The invention also provides methods to detect specific binding of a polypeptide e.g. a ligand or a receptor. The art provides numerous assays particularly useful for identifying previously unknown binding partners for receptor polypeptides of the

invention. For example, expression cloning using mammalian or bacterial cells, or dihybrid screening assays can be used to identify polynucleotides encoding binding partners. As another example, affinity chromatography with the appropriate immobilized polypeptide of the invention can be used to isolate polypeptides that recognize and bind polypeptides of the invention. There are a number of different libraries used for the identification of compounds, and in particular small molecules, that modulate (*i.e.*, increase or decrease) biological activity of a polypeptide of the invention. Ligands for receptor polypeptides of the invention can also be identified by adding exogenous ligands, or cocktails of ligands to two cells populations that are genetically identical except for the expression of the receptor of the invention: one cell population expresses the receptor of the invention whereas the other does not. The response of the two cell populations to the addition of ligands(s) are then compared. Alternatively, an expression library can be co-expressed with the polypeptide of the invention in cells and assayed for an autocrine response to identify potential ligand(s). As still another example, BIAcore assays, gel overlay assays, or other methods known in the art can be used to identify binding partner polypeptides, including, (1) organic and inorganic chemical libraries, (2) natural product libraries, and (3) combinatorial libraries comprised of random peptides, oligonucleotides or organic molecules.

The role of downstream intracellular signaling molecules in the signaling cascade of the polypeptide of the invention can be determined. For example, a chimeric protein in which the cytoplasmic domain of the polypeptide of the invention is fused to the extracellular portion of a protein, whose ligand has been identified, is produced in a host cell. The cell is then incubated with the ligand specific for the extracellular portion of the chimeric protein, thereby activating the chimeric receptor. Known downstream proteins involved in intracellular signaling can then be assayed for expected modifications i.e. phosphorylation. Other methods known to those in the art can also be used to identify signaling molecules involved in receptor activity.

4.9 THERAPEUTIC METHODS

The compositions (including polypeptide fragments, analogs, variants and antibodies or other binding partners or modulators including antisense polynucleotides)

of the invention have numerous applications in a variety of therapeutic methods. Examples of therapeutic applications include, but are not limited to, those exemplified herein.

4.9.1 EXAMPLE

One embodiment of the invention is the administration of an effective amount of the JPL polypeptides or other composition of the invention to individuals affected by a disease or disorder that can be modulated by regulating the peptides of the invention. While the mode of administration is not particularly important, parenteral administration is preferred. An exemplary mode of administration is to deliver an intravenous bolus. The dosage of JPL polypeptides or other composition of the invention will normally be determined by the prescribing physician. It is to be expected that the dosage will vary according to the age, weight, condition and response of the individual patient. Typically, the amount of polypeptide administered per dose will be in the range of about 0.01μg/kg to 100 mg/kg of body weight, with the preferred dose being about 0.1μg/kg to 10 mg/kg of patient body weight. For parenteral administration, JPL polypeptides of the invention will be formulated in an injectable form combined with a pharmaceutically acceptable parenteral vehicle. Such vehicles are well known in the art and examples include water, saline, Ringer's solution, dextrose solution, and solutions consisting of small amounts of the human serum albumin. The vehicle may contain minor amounts of additives that maintain the isotonicity and stability of the polypeptide or other active ingredient. The preparation of such solutions is within the skill of the art.

4.10 PHARMACEUTICAL FORMULATIONS AND ROUTES OF ADMINISTRATION

A protein or other composition of the present invention (from whatever source derived, including without limitation from recombinant and non-recombinant sources and including antibodies and other binding partners of the polypeptides of the invention) may be administered to a patient in need, by itself, or in pharmaceutical compositions where it is mixed with suitable carriers or excipient(s) at doses to treat or ameliorate a variety of disorders. Such a composition may optionally contain (in addition to protein or other

active ingredient and a carrier) diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration. The pharmaceutical composition of the invention may also contain cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, TNF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IFN, TNF0, TNF1, TNF2, G-CSF, Meg-CSF, thrombopoietin, stem cell factor, and erythropoietin. In further compositions, proteins of the invention may be combined with other agents beneficial to the treatment of the disease or disorder in question. These agents include various growth factors such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF), transforming growth factors (TGF-α and TGF-β), insulin-like growth factor (IGF), as well as cytokines described herein.

The pharmaceutical composition may further contain other agents which either enhance the activity of the protein or other active ingredient or complement its activity or use in treatment. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with protein or other active ingredient of the invention, or to minimize side effects. Conversely, protein or other active ingredient of the present invention may be included in formulations of the particular clotting factor, cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti- inflammatory agent to minimize side effects of the clotting factor, cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent (such as IL-1Ra, IL-1 Hy1, IL-1 Hy2, anti-TNF, corticosteroids, immunosuppressive agents). A protein of the present invention may be active in multimers (e.g., heterodimers or homodimers) or complexes with itself or other proteins. As a result, pharmaceutical compositions of the invention may comprise a protein of the invention in such multimeric or complexed form.

As an alternative to being included in a pharmaceutical composition of the invention including a first protein, a second protein or a therapeutic agent may be concurrently administered with the first protein (e.g., at the same time, or at differing times provided that therapeutic concentrations of the combination of agents is achieved at

the treatment site). Techniques for formulation and administration of the compounds of the instant application may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition. A therapeutically effective dose further refers to that amount of the compound sufficient to result in amelioration of symptoms, *e.g.*, treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to an individual active ingredient, administered alone, a therapeutically effective dose refers to that ingredient alone. When applied to a combination, a therapeutically effective dose refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

In practicing the method of treatment or use of the present invention, a therapeutically effective amount of protein or other active ingredient of the present invention is administered to a mammal having a condition to be treated. Protein or other active ingredient of the present invention may be administered in accordance with the method of the invention either alone or in combination with other therapies such as treatments employing cytokines, lymphokines or other hematopoietic factors. When co-administered with one or more cytokines, lymphokines or other hematopoietic factors, protein or other active ingredient of the present invention may be administered either simultaneously with the cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering protein or other active ingredient of the present invention in combination with cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

4.10.1 ROUTES OF ADMINISTRATION

Suitable routes of administration may, for example, include oral, rectal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections. Administration of protein or other active ingredient of the present invention used in the pharmaceutical

composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, topical application or cutaneous, subcutaneous, intraperitoneal, parenteral or intravenous injection. Intravenous administration to the patient is preferred.

Alternately, one may administer the compound in a local rather than systemic manner, for example, via injection of the compound directly into a arthritic joints or in fibrotic tissue, often in a depot or sustained release formulation. In order to prevent the scarring process frequently occurring as complication of glaucoma surgery, the compounds may be administered topically, for example, as eye drops. Furthermore, one may administer the drug in a targeted drug delivery system, for example, in a liposome coated with a specific antibody, targeting, for example, arthritic or fibrotic tissue. The liposomes will be targeted to and taken up selectively by the afflicted tissue.

The polypeptides of the invention are administered by any route that delivers an effective dosage to the desired site of action. The determination of a suitable route of administration and an effective dosage for a particular indication is within the level of skill in the art. Preferably for wound treatment, one administers the therapeutic compound directly to the site. Suitable dosage ranges for the polypeptides of the invention can be extrapolated from these dosages or from similar studies in appropriate animal models. Dosages can then be adjusted as necessary by the clinician to provide maximal therapeutic benefit.

4.10.2 COMPOSITIONS/FORMULATIONS

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in a conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. These pharmaceutical compositions may be manufactured in a manner that is itself known, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes. Proper formulation is dependent upon the route of administration chosen. When a therapeutically effective amount of protein or other active ingredient of the present invention is administered

orally, protein or other active ingredient of the present invention will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% protein or other active ingredient of the present invention, and preferably from about 25 to 90% protein or other active ingredient of the present invention. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of protein or other active ingredient of the present invention, and preferably from about 1 to 50% protein or other active ingredient of the present invention.

When a therapeutically effective amount of protein or other active ingredient of the present invention is administered by intravenous, cutaneous or subcutaneous injection, protein or other active ingredient of the present invention will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein or other active ingredient solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to protein or other active ingredient of the present invention, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art. For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration. For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from

pressurized packs or a nebuliser, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, *e.g.*, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch. The compounds may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides. In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an

emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

A pharmaceutical carrier for the hydrophobic compounds of the invention is a co-solvent system comprising benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer, and an aqueous phase. The co-solvent system may be the VPD co-solvent system. VPD is a solution of 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant polysorbate 80, and 65% w/v polyethylene glycol 300, made up to volume in absolute ethanol. The VPD co-solvent system (VPD:5W) consists of VPD diluted 1:1 with a 5% dextrose in water solution. This co-solvent system dissolves hydrophobic compounds well, and itself produces low toxicity upon systemic administration. Naturally, the proportions of a co-solvent system may be varied considerably without destroying its solubility and toxicity characteristics. Furthermore, the identity of the co-solvent components may be varied: for example, other low-toxicity nonpolar surfactants may be used instead of polysorbate 80; the fraction size of polyethylene glycol may be varied; other biocompatible polymers may replace polyethylene glycol, e.g. polyvinyl pyrrolidone; and other sugars or polysaccharides may substitute for dextrose. Alternatively, other delivery systems for hydrophobic pharmaceutical compounds may be employed. Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophobic drugs. Certain organic solvents such as dimethylsulfoxide also may be employed, although usually at the cost of greater toxicity. Additionally, the compounds may be delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various types of sustained-release materials have been established and are well known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein or other active ingredient stabilization may be employed.

The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols. Many of the active ingredients of the

invention may be provided as salts with pharmaceutically compatible counter ions. Such pharmaceutically acceptable base addition salts are those salts which retain the biological effectiveness and properties of the free acids and which are obtained by reaction with inorganic or organic bases such as sodium hydroxide, magnesium hydroxide, ammonia, trialkylamine, dialkylamine, monoalkylamine, dibasic amino acids, sodium acetate, potassium benzoate, triethanol amine and the like.

The pharmaceutical composition of the invention may be in the form of a complex of the protein(s) or other active ingredient of present invention along with protein or peptide antigens. The protein and/or peptide antigen will deliver a stimulatory signal to both B and T lymphocytes. B lymphocytes will respond to antigen through their surface immunoglobulin receptor. T lymphocytes will respond to antigen through the T cell receptor (TCR) following presentation of the antigen by MHC proteins. MHC and structurally related proteins including those encoded by class I and class II MHC genes on host cells will serve to present the peptide antigen(s) to T lymphocytes. The antigen components could also be supplied as purified MHC-peptide complexes alone or with co-stimulatory molecules that can directly signal T cells. Alternatively antibodies able to bind surface immunoglobulin and other molecules on B cells as well as antibodies able to bind the TCR and other molecules on T cells can be combined with the pharmaceutical composition of the invention.

The pharmaceutical composition of the invention may be in the form of a liposome in which protein of the present invention is combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithins, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Patent Nos. 4,235,871; 4,501,728; 4,837,028; and 4,737,323, all of which are incorporated herein by reference.

The amount of protein or other active ingredient of the present invention in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the

patient has undergone. Ultimately, the attending physician will decide the amount of protein or other active ingredient of the present invention with which to treat each individual patient. Initially, the attending physician will administer low doses of protein or other active ingredient of the present invention and observe the patient's response. Larger doses of protein or other active ingredient of the present invention may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 0.01 μg to about 100 mg (preferably about 0.1 μg to about 10 mg, more preferably about 0.1 μg to about 1 mg) of protein or other active ingredient of the present invention per kg body weight. For compositions of the present invention which are useful for bone, cartilage, tendon or ligament regeneration, the therapeutic method includes administering the composition topically, systematically, or locally as an implant or device. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone, cartilage or tissue damage. Topical administration may be suitable for wound healing and tissue repair. Therapeutically useful agents other than a protein or other active ingredient of the invention which may also optionally be included in the composition as described above, may alternatively or additionally, be administered simultaneously or sequentially with the composition in the methods of the invention. Preferably for bone and/or cartilage formation, the composition would include a matrix capable of delivering the protein-containing or other active ingredient-containing composition to the site of bone and/or cartilage damage, providing a structure for the developing bone and cartilage and optimally capable of being resorbed into the body. Such matrices may be formed of materials presently in use for other implanted medical applications.

The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. The particular application of the compositions will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalcium phosphate, hydroxyapatite, polylactic acid, polyglycolic acid and

polyanhydrides. Other potential materials are biodegradable and biologically well-defined, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are nonbiodegradable and chemically defined, such as sintered hydroxyapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalcium phosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle shape, and biodegradability. Presently preferred is a 50:50 (mole weight) copolymer of lactic acid and glycolic acid in the form of porous particles having diameters ranging from 150 to 800 microns. In some applications, it will be useful to utilize a sequestering agent, such as carboxymethyl cellulose or autologous blood clot, to prevent the protein compositions from disassociating from the matrix.

A preferred family of sequestering agents is cellulosic materials such as alkylcelluloses (including hydroxyalkylcelluloses), including methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropyl-methylcellulose, and carboxymethylcellulose, the most preferred being cationic salts of carboxymethylcellulose (CMC). Other preferred sequestering agents include hyaluronic acid, sodium alginate, poly(ethylene glycol), polyoxyethylene oxide, carboxyvinyl polymer and poly(vinyl alcohol). The amount of sequestering agent useful herein is 0.5-20 wt %, preferably 1-10 wt % based on total formulation weight, which represents the amount necessary to prevent desorption of the protein from the polymer matrix and to provide appropriate handling of the composition, yet not so much that the progenitor cells are prevented from infiltrating the matrix, thereby providing the protein the opportunity to assist the osteogenic activity of the progenitor cells. In further compositions, proteins or other active ingredient of the invention may be combined with other agents beneficial to the treatment of the bone and/or cartilage defect, wound, or tissue in question. These agents include various growth factors such as epidermal growth factor (EGF), platelet derived growth factor (PDGF), transforming growth factors (TGF-α and TGF-β), and insulin-like growth factor (IGF).

The therapeutic compositions are also presently valuable for veterinary applications. Particularly domestic animals and thoroughbred horses, in addition to humans, are desired patients for such treatment with proteins or other active ingredient of the present invention. The dosage regimen of a protein-containing pharmaceutical composition to be used in tissue regeneration will be determined by the attending physician considering various factors which modify the action of the proteins, *e.g.*, amount of tissue weight desired to be formed, the site of damage, the condition of the damaged tissue, the size of a wound, type of damaged tissue (*e.g.*, bone), the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and with inclusion of other proteins in the pharmaceutical composition. For example, the addition of other known growth factors, such as IGF I (insulin like growth factor I), to the final composition, may also effect the dosage. Progress can be monitored by periodic assessment of tissue/bone growth and/or repair, for example, X-rays, histomorphometric determinations and tetracycline labeling.

Polynucleotides of the present invention can also be used for gene therapy. Such polynucleotides can be introduced either in vivo or ex vivo into cells for expression in a mammalian subject. Polynucleotides of the invention may also be administered by other known methods for introduction of nucleic acid into a cell or organism (including, without limitation, in the form of viral vectors or naked DNA). Cells may also be cultured ex vivo in the presence of proteins of the present invention in order to proliferate or to produce a desired effect on or activity in such cells. Treated cells can then be introduced in vivo for therapeutic purposes.

4.10.3 EFFECTIVE DOSAGE

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. More specifically, a therapeutically effective amount means an amount effective to prevent development of or to alleviate the existing symptoms of the subject being treated. Determination of the effective amount is well within the capability of those skilled in the art, especially in light of the detailed

disclosure provided herein. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from appropriate in vitro assays. For example, a dose can be formulated in animal models to achieve a circulating concentration range that can be used to more accurately determine useful doses in humans. For example, a dose can be formulated in animal models to achieve a circulating concentration range that includes the IC_{50} as determined in cell culture (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of the protein's biological activity). Such information can be used to more accurately determine useful doses in humans.

A therapeutically effective dose refers to that amount of the compound that results in amelioration of symptoms or a prolongation of survival in a patient. Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio between LD_{50} and ED_{50}. Compounds which exhibit high therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. See, *e.g.*, Fingl et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1. Dosage amount and interval may be adjusted individually to provide plasma levels of the active moiety which are sufficient to maintain the desired effects, or minimal effective concentration (MEC). The MEC will vary for each compound but can be estimated from *in vitro* data. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. However, HPLC assays or bioassays can be used to determine plasma concentrations.

Dosage intervals can also be determined using MEC value. Compounds should be administered using a regimen which maintains plasma levels above the MEC for 10-90% of the time, preferably between 30-90% and most preferably between 50-90%. In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration.

An exemplary dosage regimen for polypeptides or other compositions of the invention will be in the range of about 0.01 μg/kg to 100 mg/kg of body weight daily, with the preferred dose being about 0.1 μg/kg to 25 mg/kg of patient body weight daily, varying in adults and children. Dosing may be once daily, or equivalent doses may be delivered at longer or shorter intervals.

The amount of composition administered will, of course, be dependent on the subject being treated, on the subject's age and weight, the severity of the affliction, the manner of administration and the judgment of the prescribing physician.

4.10.4 PACKAGING

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. Compositions comprising a compound of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

4.11 TRIPLE HELIX FORMATION

In addition, the fragments of the present invention, as broadly described, can be used to control gene expression through triple helix formation or antisense DNA or RNA, both of which methods are based on the binding of a polynucleotide sequence to DNA or RNA. Polynucleotides suitable for use in these methods are usually 20 to 40 bases in length and are designed to be complementary to a region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 15241:456 (1988); and Dervan et al., Science 251:1360 (1991)) or to the mRNA

itself (antisense - Olmno, J. Neurochem. 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). Triple helix-formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques have been demonstrated to be effective in model systems. Information contained in the sequences of the present invention is necessary for the design of an antisense or triple helix oligonucleotide.

4.12 DIAGNOSTIC ASSAYS AND KITS

The present invention further provides methods to identify the presence or expression of one of the ORFs of the present invention, or homolog thereof, in a test sample, using a nucleic acid probe or antibodies of the present invention, optionally conjugated or otherwise associated with a suitable label.

In general, methods for detecting a polynucleotide of the invention can comprise contacting a sample with a compound that binds to and forms a complex with the polynucleotide for a period sufficient to form the complex, and detecting the complex, so that if a complex is detected, a polynucleotide of the invention is detected in the sample. Such methods can also comprise contacting a sample under stringent hybridization conditions with nucleic acid primers that anneal to a polynucleotide of the invention under such conditions, and amplifying annealed polynucleotides, so that if a polynucleotide is amplified, a polynucleotide of the invention is detected in the sample.

In general, methods for detecting a polypeptide of the invention can comprise contacting a sample with a compound that binds to and forms a complex with the polypeptide for a period sufficient to form the complex, and detecting the complex, so that if a complex is detected, a polypeptide of the invention is detected in the sample.

In detail, such methods comprise incubating a test sample with one or more of the antibodies or one or more of the nucleic acid probes of the present invention and assaying for binding of the nucleic acid probes or antibodies to components within the test sample.

Conditions for incubating a nucleic acid probe or antibody with a test sample vary. Incubation conditions depend on the format employed in the assay, the detection methods employed, and the type and nature of the nucleic acid probe or antibody used in

the assay. One skilled in the art will recognize that any one of the commonly available hybridization, amplification or immunological assay formats can readily be adapted to employ the nucleic acid probes or antibodies of the present invention. Examples of such assays can be found in Chard, T., An Introduction to Radioimmunoassay and Related Techniques, Elsevier Science Publishers, Amsterdam, The Netherlands (1986); Bullock, G.R. et al., Techniques in Immunocytochemistry, Academic Press, Orlando, FL Vol. 1 (1982), Vol. 2 (1983), Vol. 3 (1985); Tijssen, P., Practice and Theory of immunoassays: Laboratory Techniques in Biochemistry and Molecular Biology, Elsevier Science Publishers, Amsterdam, The Netherlands (1985). The test samples of the present invention include cells, protein or membrane extracts of cells, or biological fluids such as sputum, blood, serum, plasma, or urine. The test sample used in the above-described method will vary based on the assay format, nature of the detection method and the tissues, cells or extracts used as the sample to be assayed. Methods for preparing protein extracts or membrane extracts of cells are well known in the art and can be readily be adapted in order to obtain a sample which is compatible with the system utilized.

In another embodiment of the present invention, kits are provided which contain the necessary reagents to carry out the assays of the present invention. Specifically, the invention provides a compartment kit to receive, in close confinement, one or more containers which comprises: (a) a first container comprising one of the probes or antibodies of the present invention; and (b) one or more other containers comprising one or more of the following: wash reagents, reagents capable of detecting presence of a bound probe or antibody.

In detail, a compartment kit includes any kit in which reagents are contained in separate containers. Such containers include small glass containers, plastic containers or strips of plastic or paper. Such containers allows one to efficiently transfer reagents from one compartment to another compartment such that the samples and reagents are not cross-contaminated, and the agents or solutions of each container can be added in a quantitative fashion from one compartment to another. Such containers will include a container which will accept the test sample, a container which contains the antibodies used in the assay, containers which contain wash reagents (such as phosphate buffered saline, Tris-buffers, etc.), and containers which contain the reagents used to detect the

bound antibody or probe. Types of detection reagents include labeled nucleic acid probes, labeled secondary antibodies, or in the alternative, if the primary antibody is labeled, the enzymatic, or antibody binding reagents which are capable of reacting with the labeled antibody. One skilled in the art will readily recognize that the disclosed probes and antibodies of the present invention can be readily incorporated into one of the established kit formats which are well known in the art.

4.13 SCREENING ASSAYS

Using the isolated proteins and polynucleotides of the invention, the present invention further provides methods of obtaining and identifying agents which bind to a polypeptide encoded by an ORF corresponding to any of the nucleotide sequences set forth in SEQ ID NO: 1, 2 or 3, or bind to a specific domain of the polypeptide encoded by the nucleic acid. In detail, said method comprises the steps of:

(a) contacting an agent with an isolated protein encoded by an ORF of the present invention, or nucleic acid of the invention; and

(b) determining whether the agent binds to said protein or said nucleic acid.

In general, therefore, such methods for identifying compounds that bind to a polynucleotide of the invention can comprise contacting a compound with a polynucleotide of the invention for a time sufficient to form a polynucleotide/compound complex, and detecting the complex, so that if a polynucleotide/compound complex is detected, a compound that binds to a polynucleotide of the invention is identified.

Likewise, in general, therefore, such methods for identifying compounds that bind to a polypeptide of the invention can comprise contacting a compound with a polypeptide of the invention for a time sufficient to form a polypeptide/compound complex, and detecting the complex, so that if a polypeptide/compound complex is detected, a compound that binds to a polynucleotide of the invention is identified.

Methods for identifying compounds that bind to a polypeptide of the invention can also comprise contacting a compound with a polypeptide of the invention in a cell for a time sufficient to form a polypeptide/compound complex, wherein the complex drives expression of a receptor gene sequence in the cell, and detecting the complex by

detecting reporter gene sequence expression, so that if a polypeptide/compound complex is detected, a compound that binds a polypeptide of the invention is identified.

Compounds identified via such methods can include compounds which modulate the activity of a polypeptide of the invention (that is, increase or decrease its activity, relative to activity observed in the absence of the compound). Alternatively, compounds identified via such methods can include compounds which modulate the expression of a polynucleotide of the invention (that is, increase or decrease expression relative to expression levels observed in the absence of the compound). Compounds, such as compounds identified via the methods of the invention, can be tested using standard assays well known to those of skill in the art for their ability to modulate activity/expression.

The agents screened in the above assay can be, but are not limited to, peptides, carbohydrates, vitamin derivatives, or other pharmaceutical agents. The agents can be selected and screened at random or rationally selected or designed using protein modeling techniques.

For random screening, agents such as peptides, carbohydrates, pharmaceutical agents and the like are selected at random and are assayed for their ability to bind to the protein encoded by the ORF of the present invention. Alternatively, agents may be rationally selected or designed. As used herein, an agent is said to be "rationally selected or designed" when the agent is chosen based on the configuration of the particular protein. For example, one skilled in the art can readily adapt currently available procedures to generate peptides, pharmaceutical agents and the like, capable of binding to a specific peptide sequence, in order to generate rationally designed antipeptide peptides, for example see Hurby et al., Application of Synthetic Peptides: Antisense Peptides," In Synthetic Peptides, A User's Guide, W.H. Freeman, NY (1992), pp. 289-307, and Kaspczak et al., Biochemistry 28:9230-8 (1989), or pharmaceutical agents, or the like.

In addition to the foregoing, one class of agents of the present invention, as broadly described, can be used to control gene expression through binding to one of the ORFs or EMFs of the present invention. As described above, such agents can be randomly screened or rationally designed/selected. Targeting the ORF or EMF allows a skilled artisan to design sequence specific or element specific agents, modulating the

expression of either a single ORF or multiple ORFs which rely on the same EMF for expression control. One class of DNA binding agents are agents which contain base residues which hybridize or form a triple helix formation by binding to DNA or RNA. Such agents can be based on the classic phosphodiester, ribonucleic acid backbone, or can be a variety of sulfhydryl or polymeric derivatives which have base attachment capacity.

Agents suitable for use in these methods usually contain 20 to 40 bases and are designed to be complementary to a region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1360 (1991)) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). Triple helix-formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques have been demonstrated to be effective in model systems. Information contained in the sequences of the present invention is necessary for the design of an antisense or triple helix oligonucleotide and other DNA binding agents.

Agents which bind to a protein encoded by one of the ORFs of the present invention can be used as a diagnostic agent. Agents which bind to a protein encoded by one of the ORFs of the present invention can be formulated using known techniques to generate a pharmaceutical composition.

4.14 USE OF NUCLEIC ACIDS AS PROBES

Another aspect of the subject invention is to provide for polypeptide-specific nucleic acid hybridization probes capable of hybridizing with naturally occurring nucleotide sequences. The hybridization probes of the subject invention may be derived from any of the nucleotide sequences SEQ ID NO: 1, 2, or 3. Because the corresponding gene is only expressed in a limited number of tissues, a hybridization probe derived from of any of the nucleotide sequences SEQ ID NO: 1, 2, or 3 can be used as an indicator of the presence of RNA of cell type of such a tissue in a sample.

Any suitable hybridization technique can be employed, such as, for example, in situ hybridization. PCR as described in US Patents Nos. 4,683,195 and 4,965,188 provides additional uses for oligonucleotides based upon the nucleotide sequences. Such probes used in PCR may be of recombinant origin, may be chemically synthesized, or a mixture of both. The probe will comprise a discrete nucleotide sequence for the detection of identical sequences or a degenerate pool of possible sequences for identification of closely related genomic sequences.

Other means for producing specific hybridization probes for nucleic acids include the cloning of nucleic acid sequences into vectors for the production of mRNA probes. Such vectors are known in the art and are commercially available and may be used to synthesize RNA probes *in vitro* by means of the addition of the appropriate RNA polymerase as T7 or SP6 RNA polymerase and the appropriate radioactively labeled nucleotides. The nucleotide sequences may be used to construct hybridization probes for mapping their respective genomic sequences. The nucleotide sequence provided herein may be mapped to a chromosome or specific regions of a chromosome using well known genetic and/or chromosomal mapping techniques. These techniques include in situ hybridization, linkage analysis against known chromosomal markers, hybridization screening with libraries or flow-sorted chromosomal preparations specific to known chromosomes, and the like. The technique of fluorescent in situ hybridization of chromosome spreads has been described, among other places, in Verma et al (1988) Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York NY.

Fluorescent *in situ* hybridization of chromosomal preparations and other physical chromosome mapping techniques may be correlated with additional genetic map data. Examples of genetic map data can be found in the 1994 Genome Issue of Science (265:1981f). Correlation between the location of a nucleic acid on a physical chromosomal map and a specific disease (or predisposition to a specific disease) may help delimit the region of DNA associated with that genetic disease. The nucleotide sequences of the subject invention may be used to detect differences in gene sequences between normal, carrier or affected individuals.

5. EXAMPLES

EXAMPLE 1
ISOLATION OF SEQ ID NO: 1 and 2 FROM A CDNA LIBRARIES OF HUMAN CELLS

The novel nucleic acids of SEQ ID NO: 1 and 2 were obtained from various human cDNA libraries using standard PCR, sequencing by hybridization sequence signature analysis, and Sanger sequencing techniques. The inserts of the library were amplified with PCR using primers specific for vector sequences flanking the inserts. These samples were spotted onto nylon membranes and interrogated with oligonucleotide probes to give sequence signatures. The clones were clustered into groups of similar or identical sequences, and single representative clones were selected from each group for gel sequencing. The 5' sequence of the amplified inserts were then deduced using the reverse M13 sequencing primer in a typical Sanger sequencing protocol. PCR products were purified and subjected to fluorescent dye terminator cycle sequencing. Single-pass gel sequencing was done using a 377 Applied Biosystems (ABI) sequencer. These inserts was identified as a novel sequence not previously obtained from this library and not previously reported in public databases. These sequences are designated as SEQ ID NO: 1 and 2 in the attached sequence listing, and were first disclosed in U.S. Patent Applications09/540,217 and 09/496,914.

EXAMPLE 2
ASSEMBLAGE OF SEQ ID NO: 3

The novel nucleic acids (SEQ ID NO: 3) of the invention were assembled from sequences that were obtained from various cDNA libraries by methods described in Example 1 above, and in some cases obtained from one or more public databases. The final sequence was assembled using the EST sequence as seed. Then a recursive algorithm was used to extend the seed into an extended assemblage, by pulling additional sequences from different databases (i.e. Hyseq's database containing EST sequences, dbEST, gb pri, and UniGene) that belong to this assemblage. The algorithm terminated when there was no additional sequences from the above databases that would extend the assemblage. Inclusion

of component sequences into the assemblage was based on a BLASTN hit to the extending assemblage with BLAST score greater than 300 and percent identity greater than 95%.

Using PHRAP (Univ. of Washington) or CAP4 (Paracel), a full length gene cDNA sequence and its corresponding protein sequence were generated from the assemblage. Any frame shifts and incorrect stop codons were corrected by hand editing. During editing, the sequence was checked using FASTY and/or BLAST against Genbank (i.e., dbEST version 118, gb pri 118, UniGene version 118, Genepet release 118). Other computer programs which may have been used in the editing process were phredPhrap and Consed (University of Washington) and ed-ready, ed-ext and gc-zip-2 (Hyseq, Inc.). The full-length nucleotide and amino acid sequence, are shown in the Sequence Listing as SEQ ID NOS: 3. SEQ ID NO: 3 was first disclosed in U.S. Patent Application Serial No. 09/654,936.

EXAMPLE 3
TISSUE EXPRESSION ANALYSIS OF FULL-LENGTH POLYNUCLEOTIDES OF THE INVENTION

By checking Hyseq proprietary database established from screening by hybridization, SEQ ID NO: 3 is found to be expressed in following human tissue/cell cDNA:

Library Name	No. of Positive Clones	Total No. of Clones in the Library	Tissue Origin
FBT002	**50**	**35745**	**Fetal brain**
FBR004	**18**	**27560**	**Fetal brain**
AB3001	**1**	**1565**	**Adult brain**
FBR001	**13**	**28664**	**Fetal brain**
ABT004	**9**	**31910**	**Adult brain**
ABD003	**17**	**83268**	**Adult brain**
HFB001	**14**	**74494**	**Fetal brain**
ABR006	**20**	**108204**	**Adult brain**
IBS001	**3**	**33191**	**Infant brain**
IB2002	**21**	**265743**	**Infant brain**

PIT004	**9**	**120274**	**Pituitary gland**
ABR016	**3**	**45716**	**Brain**
THA002	**2**	**32817**	**Thalamus**
IB2003	**12**	**201294**	**Infant brain**
PRT001	**1**	**28649**	**Whole organ**
UTR001	**1**	**29595**	**Uterus**
FBR006	**5**	**151893**	**Fetal brain**
NTD001	**1**	**35080**	**Neuron**
ABR008	**4**	**145661**	**Adult brain**
SUP008	**1**	**37997**	**Mixed**
FSK	**3**	**127263**	**Fetal skin**
AOV001	**6**	**259409**	**Ovary**
LYN001	**1**	**44025**	**Lymph node**
SUP015	**1**	**46850**	**Mixed**
SPC001	**1**	**61905**	**Whole organ**
MMG001	**2**	**131991**	**Mammary gland**
ECL001	**1**	**90312**	**Placenta**
THM001	**1**	**113947**	**Thymus**
CVX001	**1**	**125473**	**Cervix**
SIN001	**1**	**142562**	**Whole organ**

EXAMPLE 4

MRNA ENCODING JPL (SEQ ID NO: 5) IS HIGHLY EXPRESSED IN MELANOMA TUMORS

Figure 4 shows the relative expression of JPL mRNA that was derived from healthy tissues, and from melanoma tumors from patients.

Total mRNA derived from the melanoma tumors (H02-53T, H02-59T, H02-61T, H02-49T, H02-67T, H02-65T, H02-57T, H02-55T, H02-51T, H02-63T), tissue adjacent to the melanoma tumors (H02-52N, H02-64N, H02-60N, H02-58N, H02-56N, H02-54N,

H02-66N, H02-62N, H02-68N, H02-50N), and the total mRNA derived from normal heart, liver, lung, pancreas, skeletal muscle, kidney, spleen, small intestine, lymph node and brain was purchased from Clinomics Biosciences Inc., (Pittsfield, MA). The RNA was subjected to quantitative real-time PCR (TaqMan) (Simpson et al., Molec Vision 6:178-183 (2000)) to determine the relative expression of JPL in human tissues. The forward and reverse primers that were used in the PCR reactions were: 5' GCAGACGCCCTCCTAAAGG 3' (forward; SEQ ID NO: 11), and 5' GCTATCAGTTTGGCCATTCGA 3' (reverse; SEQ ID NO: 12), respectively. The primers were made to SEQ ID NO: 3. DNA sequences encoding Elongation Factor 1 were used as a positive control and normalization factors in all samples. All assays were performed in duplicate with the resulting values averaged.

The Y axis shows the relative fold expression of the mRNA as determined by the number of cycles required to amplify the signal from the mRNA. The larger the number of PCR cycles, the lower the amount of mRNA present in the tissue. The level of expression is reported as being relative to the lowest level detected in a sample that was set equal to 1. Absence of signal indicates complete absence of mRNA.

Figure 4 shows that JPL mRNA is expressed at high levels normal brain tissue and in melanoma tumors and in tissue adjacent to the melanoma tumors. JPL mRNA is also expressed at high levels in normal brain. Negligible expression of JPL mRNA was detected in kidney, spleen, small intestine, and lymph node, while expression of JPL mRNA was absent in skeletal muscle, pancreas, lung, liver, and heart. The results indicate that JPL polypeptide may be used as an immunotherapeutic antibody target or as a diagnostic marker for melanomas.

EXAMPLE 5

PRODUCTION OF JPL-SPECIFIC ANTIBODIES

Cells expressing JPL are identified using antibodies to JPL. Polyclonal antibodies are produced by DNA vaccination or by injection of peptide antigens into rabbits or other hosts. An animal, such as a rabbit, is immunized with a peptide from the extracellular region of JPL conjugated to a carrier protein, such as BSA (bovine serum albumin) or KLH (keyhole limpet hemocyanin). The rabbit is initially immunized with conjugated

peptide in complete Freund's adjuvant, followed by a booster shot every two weeks with injections of conjugated peptide in incomplete Freund's adjuvant. Anti-JPL antibody is affinity purified from rabbit serum using JPL peptide coupled to Affi-Gel 10 (Bio-Rad), and stored in phosphate-buffered saline with 0.1% sodium azide. To determine that the polyclonal antibodies are JPL-specific, an expression vector encoding JPL is introduced into mammalian cells. Western blot analysis of protein extracts of non-transfected cells and the JPL-containing cells is performed using the polyclonal antibody sample as the primary antibody and a horseradish peroxidase-labeled anti-rabbit antibody as the secondary antibody. Detection of an approximately 105 kD band in the JPL-containing cells and lack thereof in the control cells indicates that the polyclonal antibodies are specific for JPL.

Monoclonal antibodies are produced by injecting mice with a JPL peptide, with or without adjuvant. Subsequently, the mouse is boosted every 2 weeks until an appropriate immune response has been identified (typically 1-6 months), at which point the spleen is removed. The spleen is minced to release splenocytes, which are fused (in the presence of polyethylene glycol) with murine myeloma cells. The resulting cells (hybridomas) are grown in culture and selected for antibody production by clonal selection. The antibodies are secreted into the culture supernatant, facilitating the screening process, such as screening by an enzyme-linked immunosorbent assay (ELISA). Alternatively, humanized monoclonal antibodies are produced either by engineering a chimeric murine/human monoclonal antibody in which the murine-specific antibody regions are replaced by the human counterparts and produced in mammalian cells, or by using transgenic "knock out" mice in which the native antibody genes have been replaced by human antibody genes and immunizing the transgenic mice as described above.

EXAMPLE 6
IN VITRO ANTIBODY-DEPENDENT CYTOTOXICITY ASSAY

The ability of a JPL-specific antibody to induce antibody-dependent cell-mediated cytoxicity (ADCC) is determined *in vitro*. ADCC is performed using the CytoTox 96 Non-Radioactive Cytoxicity Assay (Promega; Madison, WI) (Hornick *et al.*, *Blood* 89:4437-4447, (1997)) as well as effector and target cells. Peripheral blood mononuclear

cells (PBMC) or neutrophilic polymorphonuclear leukocytes (PMN) are two examples of effector cells that can be used in this assay. PBMC are isolated from healthy human donors by Ficoll-Paque gradient centrifugation, and PMN are purified by centrifugation through a discontinuous percoll gradient (70% and 62%) followed by hypotonic lysis to remove residual erythrocytes. Melanoma cells (for example) are used as target cells.

Melanoma cells are suspended in RPMI 1640 medium supplemented with 2% fetal bovine serum and plated in 96-well V-bottom microtitier plates at 2 x 10^4 cells/well. JPL-specific antibody is added in triplicate to individual wells at 1 μg/ml, and effector cells are added at various effector:target cell ratios (12.5:1 to 50:1). The plates are incubated for 4 hours at 37°C. The supernatants are then harvested, lactate dehydrogenase release determined, and percent specific lysis calculated using the manufacture's protocols.

EXAMPLE 7

TOXIN-CONJUGATED JPL SPECIFIC ANTIBODIES

Antibodies to JPL are conjugated to toxins and the effect of such conjugates in animal models of cancer is evaluated. Chemotherapeutic agents, such as calicheamycin and carboplatin, or toxic peptides, such as ricin toxin, are used in this approach. Antibody-toxin conjugates are used to target cytotoxic agents specifically to cells bearing the antigen. The antibody-toxin binds to these antigen-bearing cells, becomes internalized by receptor-mediated endocytosis, and subsequently destroys the targeted cell. In this case, the antibody-toxin conjugate targets melanoma JPL-expressing cells, and delivers the cytotoxic agent to the tumor resulting in the death of the tumor cells.

One such example of a toxin that may be conjugated to an antibody is carboplatin. The mechanism by which this toxin is conjugated to antibodies is described in Ota *et al.*, *Asia-Oceania J. Obstet. Gynaecol.* 19: 449-457 (1993). The cytotoxicity of carboplatin-conjugated JPL-specific antibodies is evaluated *in vitro*, for example, by incubating JPL-expressing target cells with various concentrations of conjugated antibody, medium alone, carboplatin alone, or antibody alone. The antibody-toxin conjugate specifically targets and kills cells bearing the JPL antigen, whereas, cells not bearing the antigen, or

cells treated with medium alone, carboplatin alone, or antibody alone, show no cytotoxicity.

The antitumor efficacy of carboplatin-conjugated JPL-specific antibodies is demonstrated in *in vivo* murine tumor models. Five to six week old, athymic nude mice are engrafted with tumors subcutaneously or through intravenous injection. Mice are treated with the JPL-carboplatin conjugate or with a non-specific antibody-carboplatin conjugate. Tumor xenografts in the mouse bearing the JPL antigen are targeted and bound to by the JPL-carboplatin conjugate. This results in tumor cell killing as evidenced by tumor necrosis, tumor shrinkage, and increased survival of the treated mice.

Other toxins are conjugated to JPL-specific antibodies using methods known in the art. An example of a toxin conjugated antibody in human clinical trials is CMA-676, an antibody to the CD33 antigen in AML which is conjugated with calicheamicin toxin (Larson, *Semin. Hematol.* 38(Suppl 6):24-31 (2001)).

EXAMPLE 8

RADIO-IMMUNOTHERAPY USING JPL-SPECIFIC ANTIBODIES

Animal models are used to assess the effect of antibodies specific to JPL as vectors in the delivery of radionuclides in radio-immunotherapy to treat melanoma, hematological malignancies, and solid tumors. Human tumors are propagated in 5-6 week old athymic nude mice by injecting a carcinoma cell line or tumor cells subcutaneously. Tumor-bearing animals are injected intravenously with radio-labeled anti-JPL antibody (labeled with 30-40 μCi of ^{131}I, for example) (Behr, *et al.*, *Int. J. Cancer* 77: 787-795 (1988)). Tumor size is measured before injection and on a regular basis (i.e. weekly) after injection and compared to tumors in mice that have not received treatment. Anti-tumor efficacy is calculated by correlating the calculated mean tumor doses and the extent of induced growth retardation. To check tumor and organ histology, animals are sacrificed by cervical dislocation and autopsied. Organs are fixed in 10% formalin, embedded in paraffin, and thin sectioned. The sections are stained with hematoxylin-eosin.

EXAMPLE 9

IMMUNOTHERAPY USING JPL-SPECIFIC ANTIBODIES

Animal models are used to evaluate the effect of JPL-specific antibodies as targets for antibody-based immunotherapy using monoclonal antibodies. Human melanoma cells are injected into the tail vein of 5-6 week old nude mice whose natural killer cells have been eradicated. To evaluate the ability of JPL-specific antibodies in preventing tumor growth, mice receive an intraperitoneal injection with JPL-specific antibodies either 1 or 15 days after tumor inoculation followed by either a daily dose of 20 μg or 100 μg once or twice a week, respectively (Ozaki, *et al.*, *Blood* 90:3179-3186 (1997)). Levels of human IgG (from the immune reaction caused by the human tumor cells) are measured in the murine sera by ELISA.

The effect of JPL-specific antibodies on the proliferation of melanoma cells is examined *in vitro* using a ^{3}H-thymidine incorporation assay (Ozaki *et al.*, *supra*). Cells are cultured in 96-well plates at 1×10^{5} cells/ml in 100 μl/well and incubated with various amounts of JPL antibody or control IgG (up to 100 μg/ml) for 24 h. Cells are incubated with 0.5 μCi ^{3}H-thymidine (New England Nuclear, Boston, MA) for 18 h and harvested onto glass filters using an automatic cell harvester (Packard, Meriden, CT). The incorporated radioactivity is measured using a liquid scintillation counter.

The cytotoxicity of the JPL monoclonal antibody is examined by the effect of complements on melanoma cells using a ^{51}Cr-release assay (Ozaki *et al.*, *supra*). Melanoma cells are labeled with 0.1 mCi ^{51}Cr-sodium chromate at 37°C for 1 h. ^{51}Cr-labeled cells are incubated with various concentrations of JPL monoclonal antibody or control IgG on ice for 30 min. Unbound antibody is removed by washing with medium. Cells are distributed into 96-well plates and incubated with serial dilutions of baby rabbit complement at 37°C for 2 h. The supernatants are harvested from each well and the amount of ^{51}Cr released is measured using a gamma counter. Spontaneous release of ^{51}Cr is measured by incubating cells with medium alone, whereas maximum ^{51}Cr release is measured by treating cells with 1% NP-40 to disrupt the plasma membrane. Percent cytotoxicity is measured by dividing the difference of experimental and spontaneous ^{51}Cr release by the difference of maximum and spontaneous ^{51}Cr release.

Antibody-dependent cell-mediated cytotoxicity (ADCC) for the JPL monoclonal antibody is measured using a standard 4 h ^{51}Cr-release assay (Ozaki *et al.*, *supra*). Splenic mononuclear cells from SCID mice are used as effector cells and cultured with or without recombinant interleukin-2 (for example) for 6 days. ^{51}Cr-labeled target melanoma cells (1×10^4 cells) are placed in 96-well plates with various concentrations of anti-JPL monoclonal antibody or control IgG. Effector cells are added to the wells at various effector to target ratios (12.5:1 to 50:1). After 4 h, culture supernatants are removed and counted in a gamma counter. The percentage of cell lysis is determined as above.

EXAMPLE 10

JPL-SPECIFIC ANTIBODIES AS IMMUNOSUPPRESSANTS

Animal models are used to assess the effect of JPL-specific antibodies block signaling through the JPL receptor to suppress autoimmune diseases, such as arthritis or other inflammatory conditions, or rejection of organ transplants. Immunosuppression is tested by injecting mice with horse red blood cells (HRBCs) and assaying for the levels of HRBC-specific antibodies (Yang, *et al.*, *Int. Immunopharm.* 2:389-397 (2002)). Animals are divided into five groups, three of which are injected with anti-JPL antibodies for 10 days, and 2 of which receive no treatment. Two of the experimental groups and one control group are injected with either Earle's balanced salt solution (EBSS) containing 5-10 $\times$ 10^7 HRBCs or EBSS alone. Anti-JPL antibody treatment is continued for one group while the other groups receive no antibody treatment. After 6 days, all animals are bled by retro-orbital puncture, followed by cervical dislocation and spleen removal. Splenocyte suspensions are prepared and the serum is removed by centrifugation for analysis.

Immunosupression is measured by the number of B cells producing HRBC-specific antibodies. The Ig isotype (for example, IgM, IgG1, IgG2, etc.) is determined using the IsoDetect™ Isotyping kit (Stratagene, La Jolla, CA). Once the Ig isotype is known, murine antibodies against HRBCs are measured using an ELISA procedure. 96-well plates are coated with HRBCs and incubated with the anti-HRBC antibody-

containing sera isolated from the animals. The plates are incubated with alkaline phosphatase-labeled secondary antibodies and color development is measured on a microplate reader (SPECTRAmax 250, Molecular Devices) at 405 nm using *p*-nitrophenyl phosphate as a substrate.

Lymphocyte proliferation is measured in response to the T and B cell activators concanavalin A and lipopolysaccharide, respectively (Jiang, *et al., J. Immunol.* 154:3138-3146 (1995). Mice are randomly divided into 2 groups, 1 receiving anti-JPL antibody therapy for 7 days and 1 as a control. At the end of the treatment, the animals are sacrificed by cervical dislocation, the spleens are removed, and splenocyte suspensions are prepared as above. For the *ex vivo* test, the same number of splenocytes are used, whereas for the *in vivo* test, the anti-JPL antibody is added to the medium at the beginning of the experiment. Cell proliferation is also assayed using the ^{3}H-thymidine incorporation assay described above (Ozaki, *et al., Blood* 90: 3179 (1997)).

EXAMPLE 11

CYTOKINE SECRETION IN RESPONSE TO JPL PEPTIDE FRAGMENTS

Assays are carried out to assess activity of fragments of the JPL protein, such as the Ig domain, to stimulate cytokine secretion and to stimulate immune responses in NK cells, B cells, T cells, and myeloid cells. Such immune responses can be used to stimulate the immune system to recognize and/or mediate tumor cell killing or suppression of growth. Similarly, this immune stimulation can be used to target bacterial or viral infections. Alternatively, fragments of the JPL that block activation through the JPL receptor may be used to block immune stimulation in natural killer (NK), B, T, and myeloid cells.

Fusion proteins containing fragments of the JPL, such as the Ig domain (JPL-Ig), are made by inserting a CD33 leader peptide, followed by a JPL domain fused to the Fc region of human IgG1 into a mammalian expression vector, which is stably transfected into NS-1 cells, for example. The fusion proteins are secreted into the culture supernatant, which is harvested for use in cytokine assays, such as interferon-γ (IFN-γ) secretion assays (Martin, *et al., J. Immunol.* 167:3668-3676 (2001)).

PBMCs are activated with a suboptimal concentration of soluble CD3 and various concentrations of purified, soluble anti-JPL monoclonal antibody or control IgG. For JPL-Ig cytokine assays, anti-human Fc Ig at 5 or 20 μg/ml is bound to 96-well plates and incubated overnight at 4°C. Excess antibody is removed and either JPL-Ig or control Ig is added at 20-50 μg/ml and incubated for 4 h at room temperature. The plate is washed to remove excess fusion protein before adding cells and anti-CD3 to various concentrations. Supernatants are collected after 48 h of culture and IFN-γ levels are measured by sandwich ELISA, using primary and biotinylated secondary anti-human IFN-γ antibodies as recommended by the manufacturer.

EXAMPLE 12

DIAGNOSTIC METHODS USING JPL-SPECIFIC ANTIBODIES TO DETECT JPL EXPRESSION

Expression of JPL in tissue samples (normal or diseased) is detected using anti-JPL antibodies. Samples are prepared for immunohistochemical (IHC) analysis by fixing the tissue in 10% formalin embedding in paraffin, and sectioning using standard techniques. Sections are stained using the JPL-specific antibody followed by incubation with a secondary horse radish peroxidase (HRP)-conjugated antibody and visualized by the product of the HRP enzymatic reaction.

Expression of JPL on the surface of cells within a blood sample is detected by flow cytometry. Peripheral blood mononuclear cells (PBMC) are isolated from a blood sample using standard techniques. The cells are washed with ice-cold PBS and incubated on ice with the JPL-specific polyclonal antibody for 30 min. The cells are gently pelleted, washed with PBS, and incubated with a fluorescent anti-rabbit antibody for 30 min. on ice. After the incubation, the cells are gently pelleted, washed with ice cold PBS, and resuspended in PBS containing 0.1% sodium azide and stored on ice until analysis. Samples are analyzed using a FACScalibur flow cytometer (Becton Dickinson) and CELLQuest software (Becton Dickinson). Instrument setting are determined using FACS-Brite calibration beads (Becton-Dickinson).

Tumors expressing JPL is imaged using JPL-specific antibodies conjugated to a radionuclide, such as ^{123}I, and injected into the patient for targeting to the tumor followed by X-ray or magnetic resonance imaging.

EXAMPLE 13

TUMOR IMAGING USING JPL-SPECIFIC ANTIBODIES

JPL-specific antibodies are used for imaging JPL-expressing cells *in vivo*. Six-week-old athymic nude mice are irradiated with 400 rads from a cesium source. Three days laterthe irradiated mice are inoculated with 4×10^7 RA1 cells and 4×10^6 human fetal lung fibroblast feeder cells subcutaneously in the thigh. When the tumors reach approximately 1 cm in diameter, the mice are injected intravenously with an inoculum containing 100 μCi/10 μg of ^{131}I-labeled JPL-specific antibody. At 1, 3, and 5 days postinjection, the mice are anesthetized with a subcutaneous injection of 0.8 mg sodium pentobarbital. The immobilized mice are then imaged in a prone position with a Spectrum 91 camera equipped with a pinhole collimator (Raytheon Medical Systems; Melrose Park, IL) set to record 5,000 to 10,000 counts using the Nuclear MAX Plus image analysis software package (MEDX Inc.; Wood Dale, IL) (Hornick, *et al.*, *Blood* 89:4437-4447 (1997)).